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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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BIOLOGICAL BULLETIN

NORMAL VERSUS SUBNORMAL DEVELOPMENT IN PATIRIA MINIATA. A CAUTION TO LABORATORY EMBRYOLOGISTS.

H. H. NEWMAN.

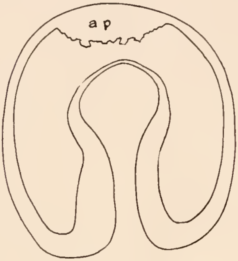
(From the Hopkins Marine Station of Leland Stanford University and the Hull Zoölogical Laboratory of the University of Chicago.)

(TWENTY-SIX FIGURES.)

Years of experience in rearing marine organisms in finger-bowls of sea water have made the present writer somewhat skeptical as to the validity of life histories worked out under these conditions. The average morphologist who is unfamiliar with the modern refinements of experimental physiology or of ecology scarcely realizes how abnormal as the environment of a marine organism is a bowl of stale and stagnant sea water kept at room temperature and in reach of direct sunlight. Such an organism is adjusted in all of its physiology to the open sea with its uniform temperature, its delicately balanced oxygen content and hydrogen-ion concentration, and its opportunities for selecting light and shade of optimum intensities.

In view of these discrepancies between the natural and the artificial environment it would seem remarkable that finger-bowl cultures ever could approximate normal development. Some organisms, however, are hardy and tolerant of suboptimal conditions and develop in finger-bowls with only slight departures from the normal. Very many marine organisms are, on the other hand, extremely sensitive to suboptimal environmental conditions and show the effects of the finger-bowl environment all too plainly.

A conspicuous example of an organism highly sensitive to suboptimal environmental conditions is the egg of the California starfish, *Patiria miniata,* with which the writer (Newman, '21 *a,* '21 *b*) has dealt at some length, calling attention to the numerous anomalies exhibited by the larvæ of this species, such as twin gastrulæ and *Bipennariæ* and numerous inhibited types. One would scarcely choose such a species as a favorable object for the study of normal development; yet this very thing has been done by Heath, who in 1917 published an account of the early development of this species.



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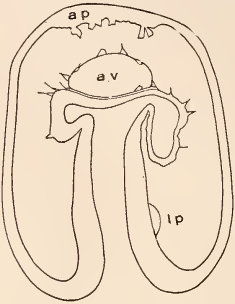

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FIG. 1. A gastrula stage, which is obviously subnormal and is to be compared with a normal gastrula such as is shown in FIG. 17. (After Heath.)

FIG. 2. A larva in the stage of cutting off the hydroenterocœl pouches. (After Heath.) *a. p.*, apical plate; *a. v.*, anterior vesicle; *l. p.*, left posterior enterocœle pouch.

Heath's account was, when it first appeared, very interesting to the present writer because of the rather startling discovery of several characters quite unknown for echinoderm larvæ, but suggestive of enteropneustan conditions. A brief account of Heath's findings must be given in order that the reader may appreciate the present contention.

In the plankton of Monterey Bay a small number of gastrulæ were found which seemed to Heath to combine in a unique way the characters of Echinodermata and of Enteropneusta. Various starfishes were artificially fertilized and reared far enough to show that the first captured plankton specimens were larvæ of *Patiria miniata*.

The artificially fertilized material was reared in finger-bowls and it was found that "the segmentation and early blastula stages do not exhibit any noteworthy features, but beyond this point certain structures arise that have no known counterpart among starfishes. The first of these unique organs is the *apical plate*. In the blastula stage the cells about the animal pole commence to elongate and, in the gastrula, form a thickened area, more or less lens-shaped in form, having approximately one half the diameter of the transverse axis of the embryo. As indicated in the drawings (Figs. 1, 2), its center is exactly opposite to the blastopore and therefore is strictly apical." Heath notes later that "all of the cells of the apical plate are packed with granules, evidently yolk."

That Heath was entirely incorrect in his interpretation of the structure called by him the "apical plate" is easily shown. The fact that the cells of this structure are packed with yolk granules should have given him a clue to the real condition; for yolk granules belong to the vegetal pole, not to the animal or apical pole. What Heath saw and accurately described were almost certainly subnormal or inhibited larvæ resulting from parthenogenetic eggs or from eggs inhibited by poor environmental conditions. These never develop normally, but always lag behind and exhibit various anomalous structures. Figures 4–12, inclusive, are camera drawings of a typical series of gastrulæ derived from parthenogenetic eggs of *Patiria*, reared by the writer in finger-bowls in the Pacific Grove Laboratory. These larvæ are all of the same age, about two days old, and are comparable with Heath's first figure (Fig.

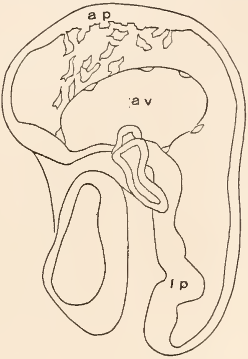

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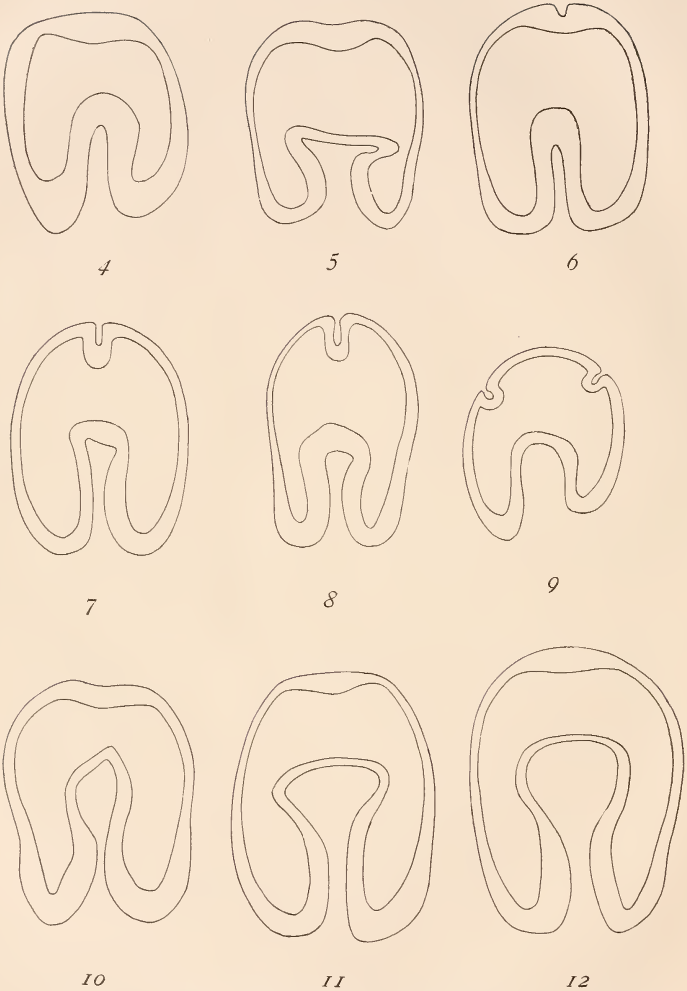
FIG. 3. A young *Bipennaria; a.p.,* "apical plate"; *a.v.,* "anterior vesicle"; *l.p.,* left posterior enterocœl. (After Heath.)

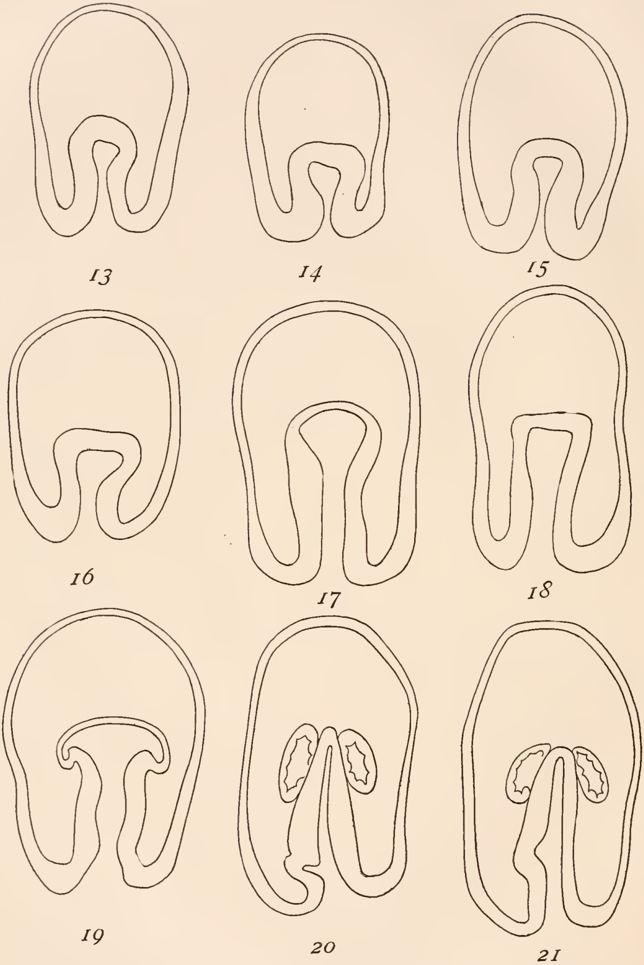


Figs. 4–12. Camera lucida outlines of optical sections of a series of *subnormal* larvæ derived by spontaneous parthenogenesis from eggs of *Patiria miniata.* In these larvæ the ectoderm in the region opposite the blastopore is frequently thickened and also very frequently invaginates to form secondary archentera. These thickenings do not occur in normal larvæ as may be seen by comparing these figures with Figs. 13–21.

1). It will be noted that the so-called "apical plate" is shown in various forms, ranging from only a slight thickening to a small secondary invagination or even several invaginations. Figure 9 shows one larva with two small secondary archentera. The physiological interpretation of this process of twinning has been given in another paper and does not especially concern us here. Sufficient to say that the so-called "apical plate" is merely a secondary region of low metabolic activity or a secondary area of primitive endoderm, as is evidenced by the presence of yolk granules, and that such a region very frequently invaginates and forms an additional archenteron.

In the light of Heath's descriptions the above sounds like a fairly dogmatic statement and one that should not be made without complete proof. The proof of the above contention is to be found in a study of the truly normal development of the species. If one artificially fertilizes a good ripe lot of *Patiria* eggs, having only a single layer of eggs on the bottom of the dish, uses just enough and not too much sperm, washes out excess sperm and any fragments of ovary or testes after an hour or so, the following conditions will be noticed within about twenty-four hours. A large percentage of gastrulæ like those shown in the camera drawings (Figs. 13 and 14) are found swimming near the surface. These are the normal larvæ and such surface larvæ rarely show any trace of an apical thickening. Near the bottom of the vessel, however, one finds numerous larvæ of the types shown in Figs. 4–9, as well as much more inhibited types. The normal larvæ swimming near the surface are perfectly typical asteroid gastrulæ and are in no way aberrant. If these larvæ are skimmed from the surface and placed in water taken fresh from the open sea, they develop as shown in Figs. 15–23, showing no differences of consequence from larvæ of other asteroids studied side by side with them.

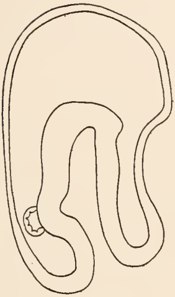
Returning once more to Heath's account, we note that he claims that "in fully 50 per cent. of the specimens in hand a few of the mesenchyme cells arising from the blind end of the archenteron, between the enterocele pouches, unite to form a small vesicle (Fig. 2, *a, v*). This usually occurs after the enterocele pouches are well differentiated, though not completely cut off." Heath seems to have seen such a thin-walled vesicle quite clearly, if one may judge



Figs. 13–21. Camera lucida outlines of optical sections of normal larvæ derived from fertilized eggs and drawn off from the surface of the dish. These normal larvæ never show "apical plate" nor "anterior vesicles." Figs. 13–15 drawn about 24 hours after fertilization. Figs. 16–18 drawn at about 48 hours. Fig. 19 drawn at 30 hours. Figs. 20 and 21 drawn at 72 hours.

by his figures. I must admit, however, that in normal material I never could see even the faintest suggestion of such a structure, while in subnormal material I occasionally observed what appeared to be a vesicle of some sort, but even in preserved material it never exhibited anything like complete continuity of outline. Heath describes this so-called *"anterior vesicle"* in great detail, dwelling on its supposed mesenchymal origin and its lack of communication with the archenteron. If, however, such a vesicle is absent in normal larvæ, discussion as to its exact structure loses significance.

A third peculiar structure is described by Heath: "a relatively



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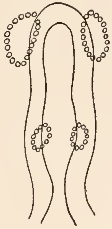

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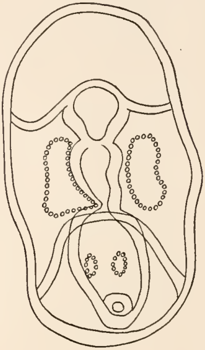

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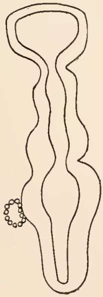

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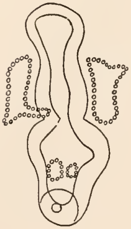

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FIG. 22. Right lateral view of larva with single posterior enterocœle vesicle. FIGS. 23, 24, 26 show larvæ with paired posterior enterocœle vesicles. FIG. 25 shows in detail the alimentary tract of a larva with but one posterior enterocœle, on the left.

large vesicle (a posterior enterocœl pouch) present on the left side only." Gemmill had described for *Asterias rubens* a pair of posterior enterocœl pouches which seemed to play little part in the formation of the posterior cœloms, but merely produced mesenchyme. My own observations of the normal *Patiria* larva enable me to confirm Gemmill's findings. More frequently than not two small paired pouches, very thin-walled, appear at about the level of the future stomach. Sometimes only one such pouch appears. The pouch is never large nor thick-walled, as one might infer from Heath's account. The nearest semblance to his structure is seen in my Figs. 20 and 21, where the archenteron shows a unilateral outpouching. Since this condition is relatively rare, however, one can not lay much emphasis upon it. I was not interested in following up the fate of any of these structures, but Heath promises that an attempt will be made to determine their ultimate fate.

On the basis of his observations on *Patiria,* Heath allows himself to engage in rather far-reaching phylogenetic speculations and sums up his position as follows: "I am strongly inclined to look upon the anterior vesicle in *Patiria* as the homologue of the proboscis cœlom of *Balanoglossus,* while the posterior outgrowth corresponds to the trunk cœlom and the intermediate pairs of vesicles in the echinoderms, often with two hydropores in certain species, is the equivalent of the collar cœlom." In another place he says that his discovery of the apical plate in *Patiria* is a strong bit of evidence in favor of the theory that the apical plate of the trochophore larva and of the echinoderm larva are homologous structures. It may readily be seen that to the phylogenist these are speculations of considerable moment, inasmuch as they seem to be confirmatory of the rather current idea that there is more than a superficial resemblance between the Tornaria larva of *Balanoglossus* and the *Bipennaria* and *Auricularia* larvæ of the starfishes and sea-cucumbers.

I have been persuaded to publish this correction of Heath's account merely to avoid the strong probability of the error creeping into our textbooks and becoming part of our permanent literature. Many another error has been thus immortalized; for, once in a textbook, a statement, no matter how erroneous, seems to be passed from generation to generation.

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'21a On the Development of the Spontaneously Parthenogenetic Eggs of *Asterina* (*Patiria*) *miniata*. Biol. Bull., Vol. 40, No. 2.

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SOMATIC MUTATIONS AND ELYTRAL MOSAICS OF BRUCHUS.

J. K. BREITENBECHER,

Contribution from the Zoölogical Laboratory of the Unlversity of Oklahoma,
Second Series, No. 10.

INTRODUCTION.

During the years 1918 to 1920 thirty-one unusual females were discovered in cultures of *Bruchus quadrimaculatus.** These unusual types were normal insects except that the elytra were of different colors. Such striking differences as these constitute what the geneticists term mosaics, and because they appear in the elytra the author describes them as elytral mosaics. It is a noteworthy fact, proven by the experiments, that these mosaics are not transmitted; it is necessary, therefore, to assume that these peculiar differences must originate in somatic tissue. It will be evident from the experiment, described herein, that these mosaics manifest an order of dominance in agreement with that of mutations. The author, therefore, concludes that these elytral mosaics are caused by somatic mutations.

It is also a noteworthy fact that these mosaics were more abundantly manifested during the period in which the author was investigating the genetics of the first three mutants (red, black, and white) which he had discovered in the progeny of the so-called "four-spotted cowpea-weevil," *Bruchus quadrimaculatus* Fabr. In a previous paper (Breitenbecher, '21) the behavior of these mutants was described and a multiple allelomorph system demonstrated for the factors which involve the four-body and elytral colors as discovered previously for *Bruchus.* Here the author proved that the order of dominance for these four-color factors was red, black, white, and tan (the wild type). The females and males of the wild type have tan elytra and bodies, while the females of the white mutant cultures have white elytra and bodies and the males have tan elytra but gray bodies. The black mutant stock shows a more pronounced dimorphism, since its females had black elytra and bodies, and its males tan elytra but grayish-black bodies. A lesser difference is seen in the red mutation, for its females had red elytra and bodies, while its males had tan elytra and reddish-gray bodies. It is evident from this description that every male had tan elytra. In conformity with an allelomorph series, the following formulæ were designated by the author: red (R R), black (R^bR^b), white (R^wR^w), and tan or wild (r r). These formulæ also indicate the order of dominance.

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* Since this paper was sent to press, 17 new mosaics have been observed, making a total of 48 to date.

TABLE I.

The Data on the Origin, Appearance, and Offspring of Elytral Mosaics in Bruchus.

Mosaic No.	Date Discovered.	Found in a Pure Culture of	Mosaic Female Elytral Color		Bred to a Male Pure for	Offspring Summary.
			Left.	Right.		
1	1918 April 20	Black	Black	Red	Black	None
2	“ May 18	“	“	“	“	“
3	“ Aug. 18	Wild	Tan	Black	Wild	All wild
4	“ Aug. 28	Black	Black	Red	Black	“ black
5	“ Sept. 21	“	Red	Black	“	None
6	“ Sept. 30	Wild	Black	Tan	Wild	All wild
7	“ Oct. 7	Black	“	Red	Black	None
8	“ Oct. 15	“	“	“	“	All black
9	“ Oct. 22	“	“	“	“	“ “
10	“ Nov. 7	“	“	“	“	“ “
11	“ Dec. 2	“	“	“	“	“ “
12	“ Dec. 12	“	Red	Black	“	“ “
13	1919 Jan. 2	“	“	“	“	“ “
14	“ Jan. 5	Wild	Tan	“	Wild	All wild
15	“ Jan. 12	Black	Black	Red	Black	“ black
16	“ Jan. 14	Wild	Tan	Black	Wild	None
17	“ Jan. 16	Black	Red	Black	Black	“
18	“ Jan. 17	Wild	Tan	“	Wild	“
19	“ Jan. 21	Black	Black	Red	Black	All black
20	“ Jan. 23	Wild	Tan	Black	Wild	None
21	“ Jan. 23	Black	Red	“	Black	None
22	“ Jan. 23	Black	Black	Red	“	All black
23	“ Jan. 25	“	“	“	“	“ “
24	“ Jan. 27	“	“	“	“	“ “
25	“ Jan. 27	“	Red	Black	“	“ “
26	“ Feb. 12	“	“	“	“	“ “
27	“ Mar. 27	“	Black	Red	“	“ “
28	“ Apr. 12	“	Red	Black	“	“ “
29	“ July 16	Wild	White	Tan	Wild	All wild
30	“ Dec. 9	“	Tan	Black	“	None
31	1920 Jan. 1	White	Black	White	White	All white

It is the purpose of this paper to describe the origin and genetic behavior of thirty-one elytral mosaic females which were discovered among thousands of normal insects examined during the progress of this work. The usual expectancy for homozygous cultures is that the elytra of the normal females will be red-red for the red mutant, black-black for the black one, white-white for the white mutant, and tan-tan for the wild type. But for these thirty-one cases, types of mosaic elytra have appeared, such as red-black, black-red, black-white, black-tan, tan-black, and white-tan. It is with these unusual types that this paper is concerned.

TABLE II.

Mosaic No.	F₁ Elytra Color.		F₂ Elytra Color.		F₃ Elytra Color.		F₄ Elytra Color.		Totals.
	♀♀	♂ Tan	♀♀	♂ Tan	♀♀	♂ Tan	♀♀	♂ Tan	
3	3 tan	3	5 tan	20	15 tan	20	21 tan	22	109
4	3 black	3	4 black	6	37 black	41	289 black	351	734
6	2 tan	2	3 tan	3	40 tan	24	120 tan	145	339
8	9 black	7	23 black	31	99 black	87	472 black	462	1,190
9	5 "	8	41 "	45	173 "	163	1,431 "	1,550	3,416
10	13 "	11	76 "	78	201 "	215	2,327 "	2,401	5,322
11	28 "	15	92 "	83	324 "	321	3,213 "	3,337	7,413
12	9 "	10	23 "	29	93 "	101	728 "	719	1,712
13	7 "	3	4 "	1					15
14	4 tan	6	13 tan	18	231 tan	239			511
15	9 black	2	10 black	8					29
19	9 "	19	83 "	91	514 black	565			1,275
22	2 "	2	21 "	28					53
23	8 "	6	139 "	174					324
24	10 "	8	3 "	7					28
25	3 "	4	97 "	93					197
26	42 "	47	317 "	323					729
27	5 "	6	98 "	91					200
28	5 "	8	202 "	224					439
29	15 tan	18	84 tan	89	327 tan	331			864
31	9 white	8	92 white	102	439 white	427			1,077

THE EXPERIMENTS.

The data and results from the breeding experiments which were carried on during the years 1918–1920 are summarized, as they bear upon the question of mosaics, in Tables I. and II. It seems easier to interpret these mosaics if one describes them in the order of their dominance (red, black, white, and tan, or the wild type).

It is evident from consulting Table I. that no mosaic has ever appeared in any pure culture of red. This fact is significant because red is dominant to black, white, and tan, and if recessive mosaics through mutations occurred in the red stock, they could not be seen. It is of interest to observe that all thirty-one elytral mosaics were females; the reason is, the colors (red, black, and white) are somatically visible in the elytra of this sex, but, on the other hand, no elytral mosaic males were discovered because the elytra of the males of the four types are always tan. Therefore, if a mosaic was produced in a male, it could not be seen, because the characters involved are sex-limited traits.

Twenty-two of these mosaics, described in Table I., originated from homozygous, black cultures. Each of these mosaic females was alike in having a normal black body, except that one elytrum was always red. Of these, eight (Mosaics 5, 12, 13, 17, 21, 25, 26, and 28) had a red, left elytrum and a black, right one, while fourteen (Mosaics 1, 2, 4, 7, 8, 9, 10, 11, 15, 19, 22, 23, 24, and 27) had a black, left elytrum and a red, right one. In order to determine whether a factor mutation had occurred, each of these mosaic insects was mated to a homozygous, black male, and the number of offspring from each pair is given in Table II. The result is the same from every mating; red was not transmitted because all progeny were pure for black. No doubt the fact that red is the only dominant to black explains why the red elytrum was visible. The fact that the black mutant cultures are more prolific and, accordingly, give rise to a greater number of offspring might account for the appearance of more mosaics, unless it is assumed that the mutation from black to red occurs more frequently.

In order to illustrate the red-black, elytral, mosaic type, let us describe Mosaic 12 (Tables I. and II.) as an example. This insect appeared on December 12, 1918, from a pure, black culture. She had a red, left elytrum and a black, right one. She was mated to a pure, black male, because her body color was, likewise, homozygous for black; her progeny were every one pure for the black character. Among the 1,712 offspring no red ones were discovered; however, red was visible in the mother because it is a dominant color to black, which is its recessive allelomorph.

From the fourteen black-red, elytral mosaics let us select Mosaic 9. This animal emerged from a culture which was homozygous for the black mutation on October 22, 1918. She possessed all the characters which were normal for a black female, except that her right elytrum was red. She was mated to a male with tan elytra, but pure for black, and this pair gave rise to 3,416 pure, black progeny. The same result is evident as before; red was not observed in any offspring, even though they were inbred for four generations; but red was visible, however, in the original parent because it is a dominant color to black.

It is next in order to consider the elytral mosaics which manifested themselves in a pure, white culture. The last mosaic (Mosaic 31, Tables I. and II.) described made its appearance on January 1, 1920, from a culture that was homozygous for the white mutant stock. Her body color and right elytrum were both normal for the white insect, but her left elytrum was black. She was bred to a male normal and pure for white. They produced 1,077 homozygous white descendants. This test indicates the same general behavior relative to dominance. Black is manifested here because it is a dominant color to white. It should be possible also to find among the progeny of a homozygous, white culture both red-white and white-red mosaics, but none were found, because it is almost impossible to keep this stock alive. This is not surprising, since only one mosaic was seen during the progress of this experiment.

Because the tan or wild type is recessive to any of the above body and elytral colors, their mosaics will next be considered. The following dominant mosaics are possible from this type: Tan-white, white-tan; black-tan, tan-black; red-tan, tan-red. Of these possible dominant mosaics three have appeared; the white-tan, tan-black, and black-tan. The others as yet have not occurred.

Thus far only one individual of the white-tan type has been discovered, Mosaic 29 (Tables I. and II.). On July 16, 1919, a remarkable elytral mosaic was found which was different from any of the others. This female had a white, left elytrum and a tan, right one; her body was normal, however, for the wild type. She originated from a wild culture, and when bred to a wild (tan) male produced 864 wild progeny. White was also visible in this elytral mosaic female because white is a somatic dominant color to tan.

This proves that white was not transmitted to any offspring; on the other hand, no tan-white insects have so far appeared.

Another kind of mosaic that was found frequently among the offspring from the wild cultures was an animal with one black elytrum. Six (Mosaics 3, 14, 16, 18, 20, and 30) of these females had tan, left elytra and black, right ones, while one (Mosaic 6) had a black, left elytrum and a tan, right one.

To illustrate the tan-black type, Mosaic 14 will be described. A mosaic female was observed after she had emerged with many other insects from a wild culture on January 5, 1919. This female had a normal, tan body and tan, left elytrum, while her right elytrum was black. When mated with a normal (tan) wild male, her offspring produced through three generations 511 tan or wild descendants. This type of a mosaic proves that black was not transmitted, because it did not appear in any offspring; again black appeared in this insect because black is a dominant to tan, its recessive allelomorph.

Of the black-tan, Mosaic 6 is the only one which was observed. She was found in a culture from wild parents on September 30, 1918. Her body and right elytrum were both tan, but her left elytrum was black. She was mated to a wild male and produced 339 homozygous, wild progeny. The result shows that the black visible in this mosaic was not transmitted because it is a somatic dominant to tan (the wild type).

Discussion.

The result of the experiments previously described shows that these somatic modifications in *Bruchus,* which concern the elytra of the females, are not due to factor mutations in the germ cells, because they are not transmitted. It seems, therefore, that the best interpretation is to assume that they are somatic mutations. Ordinary somatic variations are modifications that are supposed to be caused by environmental factors, as they are not inherited. They always display a normal variability curve. The converse of this is true, for these mosaics, since they manifest a sharp discontinuous variation, not unlike a mutation. Mutations have usually been described as discontinuous variations that breed true. A mutation that occurred in a germ cell in an animal would breed true because of the necessity of sexual reproduction, but a mutation that took place in a somatic cell could be discontinuous, although it would not be transmitted sexually. This reasoning led the author to suggest that these elytral mosaics in *Bruchus* should be called somatic mutations.

In plants mutations may occur in the germ cells or in any meristematic tissue. According to Babcock and Clausen ('18), "Factor mutations in meristematic cells, or vegetative mutations, as distinguished from those originating in the germ cells, give rise to simple *bud sports* or to *chimeras* according to the location of the mutating cells. A bud sport is a shoot or branch which differs genotypically in one or more characters from the remainder of the plant. Here the factor mutation must occur in one of the undifferentiated cells of the very young shoot. Just as in the case of factor mutations in germ cells, so in vegetative mutations the somatic effects range from single visible character differences to manifold effects in which many structural details are different." The fact that somatic factor mutations do occur in plants seems to be well established, and, furthermore, these somatic factor mutations display the same order of dominance, as manifested by factor mutations, which occur in germ cells. Although somatic factor mutations are extremely rare in animals as compared to this same behavior in plants (because the latter can be propagated, while the former can not be), this, in itself, is not sufficient argument to discredit the idea that the same phenomena are involved. It appears, therefore, in this relation that the mosaics in *Bruchus* are not unlike somatic mutations in plants. Emerson ('21) has lately noted similar phenomena in maize.

The ordinary gynandromorph, as described by Morgan and Bridges ('19), is an animal that shows some male characters on one side of the body and female on the other, but the elytral mosaics in *Bruchus* are not gynandromorphs in this sense because the sexual organs are not modified, every mosaic being a normal female. These authors ('19) further showed that sex-linked characters are usually involved in the gynandromorphs of *Drosophila.* There is evidence that nearly all gynandromorphs are potentially females, but that a sex-mosaic results through the elimination of one sex-chromosome immediately after fertilization; this is due to the fact that the normal female in *Drosophila* has two (XX) sex-chromosomes in her body, but if one is eliminated on one side of her body, male characters will then develop on that side. There is in *Drosophila* no essential difference between gynandromorphs (sex-mosaics) and somatic mosaics, except that the former concern the elimination of one sex-chromosome, while the latter involve the elimination of an autosome. The mechanism of elimination, too, is the same.

It is true of *Bruchus* that all visible mosaics were females, but the author ('21) has shown that these color factors in this insect are not sex-linked, but are sex-limited, autosomal traits. It is improbable that any change in the X-chromosome could produce these mosaics. Morgan and Bridges ('19) have shown that gynandromorphs are neither caused by partial fertilization, as first suggested by Boveri, nor due to polyspermy, as first interpreted by Morgan, but are caused by chromosome elimination. Chromosome elimination, as described by the above writers, means that during some early stage of development of the embryo one of the daughter sex-chromosomes fails to pass over to the daughter plate, and thereby becomes eliminated from the nucleus. Relative to the mosaics of *Bruchus,* it is impossible to account for their appearance through the elimination of the X-chromosome, because these characters involve the autosome in which the allelomorph series for the R (red) gene is located, and not the X-chromosome.

Morgan and Bridges ('19) account for mosaics of *Drosophila* by chromosomal elimination; in one case they tried to account for the origin of a mosaic, as produced from binucleated eggs, because each paternal nucleus was known to have had a different ancestry. It is unnecessary to make this latter assumption for these mosaics of *Bruchus,* because both nuclei of a binucleated egg (if such could occur) would of necessity be alike, and when fertilized by a normal sperm, all of the offspring would be homozygous, unless it is assumed that a mutation occurred in one nucleus before fertilization, and in that event the effect would not be localized in a single wing, but would involve half the entire body.

In *Drosophila* nearly all mosaics can be interpreted as caused by chromosome elimination, because the homologous chromosome from one parent carries factors that are different from its mate.

If these mosaics of *Bruchus* had originated from heterozygous cultures, then the elimination of one autosome might produce one elytrum of one color and the other of a different one; but since these mosaics came from homozygous cultures, it is evident that the elimination of an autosome previous to or even after fertilization could not have this effect. It is difficult to conceive of any kind of autosomal non-disjunction that could possibly create a red elytrum, for example, when the insect was pure for black. Neither can non-disjunction nor the elimination of an autosome have any effect in causing this kind of a mosaic unless one assumes that a mutation occurs after fertilization. The evidence is in favor of this interpretation because these mosaics are not transmitted. It appears, therefore, more reasonable to assume that a mutation has occurred in one autosome during the embryonic development of the insect. Neither is it necessary to assume that autosome elimination or non-disjunction is essential to account for these mosaics even after a somatic mutation has taken place, unless these mosaics were recessives instead of dominants.

Normally recessive mosaics could not be seen, even though they occurred frequently, because a recessive autosome mutation could not be visible in the F_1 progeny, except through autosome elimination. To illustrate the appearance of such a mosaic, let us suppose in a pure culture for red that a female was found with one black elytrum (black is recessive to red). The normal autosome complex on each side of the body of a homozygous red female would be R R. If a mutation to black would occur in one autosome on her right side (R^b is the formula for black), then her right side would be R R^b, and her left side, normal, R R; but because red is dominant to black, when heterozygous, she would still appear red unless the autosome carrying R (red) on her right side would be eliminated, then one side would be black and the other red.

The time in the ontogeny at which the mutation occurred would govern the extent of its effects. If it took place at the first cleavages, for instance, the entire half of the body might be affected; but if it took place later at the time when the *anlagen* of the wings are differentiated, then one elytrum would be black and the other red.

It is true, however, for every mosaic so far discovered in

Bruchus that only elytral colors which are dominant to it have been found, so it is essential to apply next the elimination mechanism to the actual mosaics observed in this insect.

The most common mosaic in this beetle is a red elytrum and a black body originating from pure black stock. This condition is accounted for by assuming a mutation to red in one autosome. Let us now suppose in a normal female, homozygous for black, that, previous to the formation of an elytrum, one autosome mutated to red; the autosomes for the normal elytrum, therefore, would be R^bR^b (pure black), while the mosaic elytrum autosomes R^bR (heterozygous for black and red). Since red is dominant to black, the one R on the one side of this insect is sufficient to make this elytrum red; therefore, a mosaic results as was observed for *Bruchus*. (This is true for the thirty-one mosaics found.) Now, let us apply autosomal elimination for this mosaic; if the autosome R^b (black) was eliminated, the proper mosaic would result; but, on the other hand, if the autosome carrying R (red) was eliminated, the insect would not be a mosaic, but a normal, homozygous, black individual.

Again, if the mutation occurred in two autosomes (a very improbable assumption), it could cause the following mosaic from a homozygous, black insect. Supposing the autosomes on the right side of its body are normal, R^bR^b (black right elytrum), but on the left side a mutation to red occurred in each autosome, then the autosomes for its right elytrum would be R R (pure red). This insect would then have a black right elytrum and a red left one; in this case elimination could not change the mosaic.

The first interpretation with reference to dominant autosome mutations appears correct for these mosaics of *Bruchus*. It seems, therefore, that to account for these mosaics autosomal elimination is not essential, unless a mosaic is visible which is recessive to the normal; in that case autosome elimination would be necessary.

The most plausible explanation, then, is to regard these thirty-one dominant, elytral mosaics of *Bruchus* as somatic mutations that originate in an autosome on one side of the body some time during its ontogeny. The results as described in this paper for *Bruchus* agree with Morgan and Bridges ('19) relative to dominant mutations in somatic tissue. "The general evidence from mutations in

Drosophila," to quote these authors, "makes it highly probable that when a mutation occurs it takes place in only one chromosome of the pair. Hence any mutation in somatic tissue, if recessive, would be concealed by the presence of the normal allelomorph in the homologous chromosome." This is the key to the situation for *Bruchus,* since these thirty-one mosaics appear through dominant somatic mutations in one chromosome of the pair.

It is evident that these somatic mutations in *Bruchus* concern the autosome in which the multiple allelomorph gene for R (red) is located. The difference between the factor mutations which the author ('21) discovered for the body and elytral color factors located at the locus, R, in this autosome and its somatic mutations as manifested in these elytral mosaics is that the former are transmitted, while the latter are not.

The evidence further indicates that there is a chromosome continuity between the gene for R (red) in this autosome in the germ cell and this same gene as manifested through the thirty-one mosaics in *Bruchus.* Of these somatic mutations, twenty-two occurred in homozygous black cultures, through a mutation from recessive black to dominant red; only one from a pure white culture, through a mutation from recessive white to dominant black; while eight appeared from wild stock, seven of these mutated from recessive tan to dominant black, and one of these from recessive tan to dominant white.

The most noteworthy result is that germinal mutations and somatic mutations are identical factor mutations, because both originate through a mutation in a chromosome. This paper furnishes evidence in favor of the chromosome hypothesis.

The conditions noted in these unilateral somatic mosaics perhaps suggest that the chromosome mechanism may also be found to account more generally for the bilateral type of body symmetry, at least in certain groups. Conklin has pointed out that while types of cleavage, symmetry, etc., are determined by cytoplasmic influences, this is not at variance with the chromosome doctrine, since the cytoplasm is itself influenced by the nucleus through the large amount of nuclear material which escapes at every mitosis. The investigations regarding cytoplasmic localization and their part in inheritance merely shows "that in early development inherited characteristics, like material substances, are chiefly derived from the mother." The facts upon which are based the conclusions recently drawn from a study in asymmetry in *Peromyscus* by Sumner and Huestis ('21) are not necessarily contradictory to Conklin's point of view, in spite of their statement that "the chromosome mechanism of heredity . . . is ill-adapted to account for the transmission of definite spatial relationships. . . ." Future evidence bearing upon the question of symmetry will be very welcome.

SUMMARY AND CONCLUSIONS.

1. During the years 1918 to 1920, among the thousands of Bruchid insects examined, thirty-one elytral mosaic females were found in homozygous cultures of the black, white, and tan stocks. The usual expectancy from pure cultures is that the elytra of the normal female will be red-red for the red mutant, black-black for the black one, white-white for the white mutant, and tan-tan for the wild type, but such mosaic types as red-black, black-red, black-white, black-tan, tan-black, and white-tan have appeared from homozygous cultures. These display the same order of dominance as was discovered by the author ('21) for the four body colors (red, black, white, and tan or the wild type).

2. Chromosome elimination of any kind is not essential to account for these mosaics, but a dominant somatic mutation in one chromosome of the pair is the most plausible explanation. The time in the ontogeny at which the mutation occurred would govern the extent of its effects; the earlier that it took place, the greater its effect. It is evident that it did occur at the time when the *anlagen* of the wings were differentiated, otherwise an elytral mosaic would not have appeared.

3. The difference between the factor mutations which the author ('21) discovered for the body and elytral color factors located at the locus R (red), in an autosome and its somatic mutations as manifested in these elytral mosaics, is that the former are transmitted, while the latter are not. There appears to be a somatic continuity, however, between this gene for R (red) in this autosome in the germ cell and this same gene as manifested through its thirty-one somatic mutations.

4. Of these thirty-one somatic mutations, twenty-two occurred through a mutation from homozygous, recessive black to dominant red; one through a mutation from homozygous, recessive white to a dominant black; while eight originated in wild cultures, seven of these mutated from recessive tan to dominant black, and one of these from recessive tan to dominant white.

5. In conclusion, the most noteworthy result is that somatic and germinal mutations are identical in that both are due to mutations originating in a chromosome. The time in ontogeny at which the mutation occurs determines whether it appears as germinal or merely as somatic.

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NOTES ON PSITHYRUS, WITH RECORDS OF TWO NEW AMERICAN HOSTS.[1]

O. E. PLATH,

Massachusetts Institute of Technology, Cambridge, Mass.

In Franklin's monumental work, "The Bombidæ of the New World," we have a taxonomic outline of the American bumblebees, equal, if not superior, to similar works on the European species. One is rather disappointed, however, on comparing our knowledge, or rather lack of knowledge, concerning the habits of the American species with the interesting accounts of the habits of the European species given by men like Hoffer and Sladen. What we do know about the habits of our North American species we owe chiefly to the efforts of Putnam, Franklin, and Frison, but the field is so large that the surface has hardly been scratched. This is especially true of one of the subdivisions of our Bremidæ,[2] the genus *Psithyrus*. Of the 13 or 14 species of *Psithyrus* described from the New World, the hosts of only two have thus far been recorded. On July 7, 1914, Sladen ('15), at Agassiz, British Columbia, dug up a nest of *Bremus flavifrons* Cresson, victimized by *Psithyrus insularis* Smith, and two years later Frison ('16) recorded that, during the summers of 1910 and 1915, he had repeatedly found the nests of *Bremus pennsylvanicus* De Geer infested by *Psithyrus variabilis* Cresson. To these two records the writer wishes to add two others, those for *Psithyrus laboriosus* Fabricius and *Psithyrus ashtoni* Cresson.

Before going into detail, however, it seems desirable to give a brief résumé of the structure, life history, and habits of these social parasites, as we know them chiefly from the work of Kirby (1802),

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[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 201.

[2] In using *Bremus* and *Bremidæ* instead of the familiar *Bombus* and *Bombidæ*, the writer follows Frison ('19) who in a recent paper pointed out the reasons for making this change (cf. also the extensive paper by Morice and Durrant ('14) on this subject).

Smith ('55), Hoffer ('81, '88), Sladen ('99, '12, '15), and Frison ('16, '21).

STRUCTURE.

Both sexes of the genus *Psithyrus* so closely resemble those of the genus *Bremus* that anyone, other than a specialist, would see no difference between them; and even the specialist sometimes has difficulty in determining whether a certain male is a *Bremus* or a *Psithyrus,* so that it has occasionally happened that *Bremus* males have been described as *Psithyri,* and *vice versa.* It is quite easy, however, to tell a *Bremus* female from a *Psithyrus,* because the latter lacks *corbiculæ,* or pollen baskets, a fact which was first noticed by Kirby (1802, I., pp. 209, 210). Illiger (1806, p. 173), suspecting a corresponding difference in habits, separated them from the true bumble-bees, and Newman ('34, p. 404) later gave the group the generic name of *Apathus.* This term held sway for over 40 years, when it was discovered that the name *Psithyrus* had been given to the group by Lepeletier in 1832 (p. 273).

LIFE HISTORY AND HABITS.

According to Hoffer ('88) and Sladen ('12), the young *Psithyrus* queen, like the young *Bremus* queen, hibernates in the ground, but reappears somewhat later in spring than does the latter. Having no apparatus for collecting pollen, she is unable to found a colony of her own, as does the *Bremus* queen, but, like the European cuckoo and some of our American cowbirds, perpetuates her kind by entering, and laying her eggs in, the nests of her more industrious cousins of the genus *Bremus.* The latter, like the dupes of the European cuckoo, rear the larvæ of these lazy guests instead of their own. As will be seen later, these latter, at least those in the earlier stages of development, are probably systematically destroyed by the *Psithyrus.*

That the *Psithyrus* queen does not always gain admittance to a *Bremus* colony without a struggle is indicated by the frequent discovery in bumble-bee nests of dead or disabled *Psithyri* or *Bremi,* or both, and is confirmed by direct observation when a *Psithyrus* first enters, or is placed in, a *Bremus* nest. In these encounters the *Psithyrus* has a great advantage over the individual members of the *Bremus* colony. She has a powerful sting, and her integument is so thick that her opponents are unable to penetrate it with their stings. But, like Siegfried and Achilles, the *Psithyrus* is vulnerable in certain places—*e.g.,* the neck—and it is chiefly for this reason that she is not always successful.

However, as already indicated, a *Psithyrus* queen sometimes does gain admittance to a *Bremus* colony without losing her life and may remain with the colony until she dies. Here the question arises: What is the attitude of such a *Psithyrus* queen and the rightful owners of the nest to each other? The two foremost European authorities on the subject do not agree on this point. Hoffer ('81, '88), who studied the habits of the European *Psithyri* more extensively than any other investigator, repeatedly found the *Psithyrus* queen living peacefully with the members of the *Bremus* colony, including the host queen. Sladen ('99, '12, '15), on the other hand, claims that the *Psithyrus* queen, at least those of *Psithyrus rupestris* Fabricius and *Psithyrus vestalis* Foucrier, always kills the *Bremus* queen,[1] either at the time the *Psithyrus* queen enters the nest or a few days later, when the *Psithyrus* is about to begin egg-laying. After the *Psithyrus* has committed this murder, the *Bremus* workers, according to Sladen ('12), constantly watch for an opportunity to avenge their mother by killing the usurper. He (p. 60 ff.) very vividly describes this phase in the life history of the *Psithyrus* queen and the afflicted bumble-bee colony as follows: "It is the practice of the *Psithyrus* female to enter the nest of the *Bombus,* to sting the queen to death, and then to get the poor workers to rear her young instead of their own brothers and sisters.

"The way in which the *Psithyrus* queen proceeds in order to ensure the success of her atrocious work has all the appearance of a cunning plan, cleverly conceived and carried out by one who not only is a mistress of the crime of murder, but also knows how to commit it at the most advantageous time for herself and her future children, compelling the poor orphans she creates to become her willing slaves. . . . Her first care is to ingratiate herself with the inhabitants, and in this she succeeds so well that the workers soon cease to show any hostility towards her. Even the queen grows accustomed to the presence of the stranger, and her alarm disappears, but it is succeeded by a kind of despondency. Her interest and pleasure in the brood seem less, and so depressed is she that one can fancy she has a presentiment of the fate that awaits her. It is by no means a cheerful family, and the gloom of impending disaster seems to hang over it."

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[1] On July 14, 1911, Sladen ('12, p. 70) took a nest of *Bremus hortorum* Linnæus, containing 49 workers of *B. hortorum,* and 16 young queens and 2 males of *Psithyrus barbutellus* Kirby. From this rather meager evidence he concludes that *Ps. barbutellus* is probably "parasitic in the same deadly way as *Ps. rupestris* and *vestalis.*"

The hostile attitude of the *Bremus* workers toward the *Psithyrus* which has killed their mother (in one case weeks before) is described by Sladen ('12, pp. 256, 277) as follows: "It was clear that the workers deposed the *Psithyrus* queen, and I think that this was the culminating act in a revolt that the queen had all along found it difficult to repress. . . . It appears that when the [*Bremus*] colony is populous the [*Psithyrus*] *rupestris* [queen] will lose her life unless she maintains constantly her rule of repression."

Though interesting, this account of the behavior of the *Psithyrus* queen and the victimized *Bremus* colony toward each other is not only opposed by the earlier work of Smith ('55, p. 210) and Hoffer ('81, '88), but also by recent observations on the habits of some of our American *Psithyri.*

During the summer (June 24 to August 12) of 1921 the writer located 14 *Bremus* colonies, 13 of which were placed in observation boxes a day or two after they were discovered. They were then transferred to the Bussey Institution, where they were kept under observation for periods varying from one to four months. Each box was provided with a glass cover and an opening which communicated with the outside world through holes in boards placed below the screens of three of the windows (one on the second and two on the third floor) of one of the Bussey buildings. Nearly all of the colonies flourished, some producing hundreds of young queens and males. On August 9, colony No. 13 (*B. affinis*) was dug up after much effort, the tunnel being over seven feet in length. The nest was about three feet below the surface and contained the old queen and about 100 workers of *Bremus affinis* Cresson, and the old queen, 3 young queens, and 6 males of

Psithyrus ashtoni. The comb consisted of numerous empty *Bremus* and *Psithyrus* cocoons and a large quantity of *Psithyrus* brood in various stages of development. This colony was kept under observation until September 26. From August 9 until September 14 the nest was examined at least twice a day. Once a week every worker was caught, so that the young *Psithyri,* which had hatched during the preceding week, could be collected without incurring the wrath of the colony. Thereupon the young *Psithyri* were placed in a box and fed with honey and pollen until their pile attained its full color. In this way 29 males and 61 females of *Ps. ashtoni* were collected from this *Bremus-Psithyrus* colony, but a considerable number of males, and perhaps a few females, probably made their escape during the last few days preceding the weekly collections.

Despite the fact that the abdomen of the *Bremus* queen was much distended, and that she was seen to lay eggs on August 10 and on subsequent days, not a single *Bremus* queen, male, or worker hatched. As the nights became colder during the early part of September, the *affinis-ashtoni* colony, which by this time had dwindled down to about a dozen individuals, gradually died off. On September 12 the old *Psithyrus* queen was found dead in the nest, two days later the *Bremus* queen was missing, and the last worker died on September 26.

In this case the *Psithyrus* queen must have gained admittance to the *affinis* nest during or before the first few days of July. The *Psithyrus* and the *Bremus* queen, therefore, lived together for at least two months. During the time they were under observation (August 9 to September 12) the two queens did not show the least antagonism, nor did any of the other members of this *Bremus-Psithyrus* colony exhibit the slightest sign of hostility toward each other.

The fact that no young bees developed from the eggs laid by the *Bremus* queen corroborates similar observations by Hoffer ('88)[1] and Sladen ('12). Here the question arises: What becomes of the eggs laid by the *Bremus* queen, or workers, of a *Psithyrus*-ridden nest? Hoffer ('88) considers it probable that the young larvæ which hatch from them are eaten by the young *Psithyrus* larvæ. However, Sladen ('12) actually saw a *Psithyrus* queen devour the eggs of *Bremus* workers, and believes that she always disposes of them in this way. He (p. 257) also thinks it is probable that the race-suicidal habit of the workers of *Bremus lapidarius* Linnæus, which sometimes eat the newly laid eggs of their mother, is associated with the parasitism of *Psithyrus*. He believes that the workers which devour the eggs of their stepmother perpetuate this instinct through their sons. In support of this theory, he states that he has never seen the workers of *Bremus latreillellus* Kirby, "a species that is not preyed upon by any species of *Psithyrus*," molest their mother's eggs. This explanation does not seem plausible. It is a well-known fact (cf. Wheeler, '10) that both queen and worker ants, even of those species which are not molested by parasitic ants, sometimes eat their own eggs. Moreover, the writer has frequently seen the workers of *Bremus fervidus* Fabricius eat their mother's eggs, and this species, as will be shown in another paper, probably does not suffer any species of *Psithyrus* to breed in its nests, a view which is supported by more than twenty records (10 by Putnam, "a large number" by Franklin, and 7 by the writer) of *fervidus* nests, none of which were victimized by a *Psithyrus*.

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[1] This author ('88, p. 101) reports two exceptions to this rule. On September 1, 1880, he took home a nest of *Bremus variabilis* Schmiedeknecht containing the old queen and 15 workers of *B. variabilis,* and 8 females and 10 males of *Psithyrus campestris* Panzer. During the next few days, 4 more males and 9 females of *Ps. campestris,* and 2 males and 3 females of *B.*

The question has sometimes been raised as to whether or not the members of the genus *Psithyrus* have any workers. Hoffer ('88, p. 114) answers this question negatively. Among the 61 *ashtoni* females reared by the writer there is a great variation in size (cf. Plate I.), some specimens not exceeding that of medium-sized *affinis* workers. It will also be noticed (cf. Plate I.) that the *Psithyrus* males vary greatly in size. If it is true that the difference between queen and worker of the social *Hymenoptera* is due to a quantitative or qualitative difference in feeding during the

variabilis hatched from this colony. Four years later (June 24, 1884), one of his sons discovered a nest of *Bremus pratorum* Linnæus, containing the old queen, 2 young queens, 5 males, and 26 workers of *B. pratorum*, and the old queen, 9 young queens, and 4 males of *Psithyrus quadricolor* Lepeletier.

larval stage, then these small *Psithyrus* females are comparable to *Bremus* workers. Whether or not they are more inclined to work than the full-sized *Psithyrus* females remains to be determined.

Sladen ('12, pp. 62, 63) believes that the *Psithyrus* queen, like a dog, is largely guided by scent in locating the nests of her victims, and is much more likely to find them when they have a short tunnel. In support of this view, he points out that most of the nests containing *Psithyri* which he dug up had tunnels not exceeding fifteen inches in length, and that in no case were they more than two feet long, and he thinks it is probable that species like *Bremus terrestris* Linnæus and *Bremus lapidarius* often have longer tunnels than other species in order to escape the *Psithyrus*. That this precaution is no absolute insurance against the invasion of *Psithyrus ashtoni* is indicated by the *affinis-ashtoni* nest with a tunnel of more than seven feet.

In a recent paper Frison ('19), on the basis of geographical distribution, expressed the belief that *Ps. laboriosus* "is an inquiline in the nests of *B. fervidus* or *B. vagans*." The writer is pleased to be able to confirm the last part of this prediction. On August 2, colony No. 11 (*B. vagans*) was dug up and transferred to a third-story window in one of the Bussey buildings. Eight days later (August 10) the writer found that a *Ps. laboriosus* queen had gained admittance to the nest. At this time the *vagans* colony consisted of the old queen, about 50 workers, and several males. In the afternoon of the same day the *Psithyrus* queen was observed in the act of tearing open a mass of wax containing small *Bremus* larvæ, so that three of the latter rolled to the bottom of the nest box, leaving the remaining four widely exposed. As usual, these larvæ were thrown out of the nest a few minutes later by the *Bremus* workers. About 5 P.M. the *Psithyrus* queen began to gather wax from the cocoons and built a small cell in which, about 7 P.M., she laid several eggs. As each egg was deposited the sting of the *Psithyrus* penetrated the wall of the cell.

Hoffer ('88), who, despite his extensive observations, never saw a *Psithyrus* oviposit, states (p. 100) that the *Psithyrus* queen lays her eggs in pollen masses in which *Bremus* eggs, or larvæ, are already present. This, as is shown by the observations of Sladen ('12) and of the writer, is not true of *Ps. rupestris* and *Ps. labori-*

osus, and, judging from the oviposition of the members of the genus *Bremus,* is rather improbable of any of the *Psithyri.* The observations of Sladen ('12) and of the writer also indicate that Hoffer's ('88) surmise that the *Psithyrus* larvæ, at least those recently hatched, devour the larvæ of their host,[1] is not true of all, if of any, members of the genus *Psithyrus.*

Six of the eggs laid by the *Ps. laboriosus* queen developed into queen larvæ which spun their cocoons about August 25. On September 7, a *Ps. laboriosus* queen emerged from one of these. The queens in the other five cocoons were heavily parasitized by *Melittobia* sp. and did not hatch. Nor did any of the *Bremus* eggs and larvæ develop which were present in the nest when the *Psithyrus* queen gained admittance to this colony. Whether or not, in this case, the *Bremus* queen laid any eggs[2] after the appearance of the *Psithyrus,* could not be determined.

Hoffer ('88, pp. 104, 105) states that whenever the old *Psithyrus* queen dies, while her larvæ are still very young, the latter, as a rule, likewise perish, and he considers it probable that the *Psithyrus* queen forages and contributes something to the support of her offspring, at least during their early larval stages. The latter is not true of *Psithyrus ashtoni,* and, as the observations of Sladen ('12, p. 65) and of the writer indicate, is also improbable of other species. Both the *Ps. laboriosus* and the *Ps. ashtoni* queen were always at home, and, as regards the latter, foraging was out of the question. She was able to fly but a few feet at the time the *affinis-ashtoni* colony was taken, and still her brood, including some newly hatched larvæ, was in a flourishing condition. It is probable, therefore, that the *Psithyrus* larvæ which Hoffer ('88) had under observation died from other causes.

The behavior of the *Psithyrus laboriosus* queen and the *vagans* colony to each other was the same as that of the members of the *affinis-ashtoni* colony, except during the first three or four days.

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[1] This is true of the larvæ of certain solitary parasitic bees, as Verhoeff ('92), Höppner ('04), and Graenicher ('05) have shown.

[2] Sladen ('12, p. 69) claims that a large number of workers in a *Psithyrus*-ridden colony become fertile. This was true of my queenless *B. impatiens* colonies, but none of these would accept a *Psithyrus* queen. However, in my *Psithyrus*-infested colonies, all of which possessed the old *Bremus* queen, none of the workers took to egg-laying.

On the first day (August 10) the *laboriosus* queen seized nearly every worker with which she came in contact and rolled the latter toward the ventral side of her abdomen, and made movements as if to sting her victim. This mauling, as a rule, lasted for only a few seconds, when the worker, which in every case was absolutely passive, was again released. In seizing the workers, both the mandibles and the first pair of legs were usually employed simultaneously, but on one occasion a worker was first lifted up by the pile of its thorax with the mandibles and then rolled below the body of the *Psithyrus*. None of the workers seemed to be any the worse for this mauling.[1]

On the second day (August 11) the *Psithyrus* queen only occasionally seized a worker and treated it in the manner described. On the third day (August 12) this rough treatment of the *Bremus* workers became still less frequent, and thereafter the behavior of the *Psithyrus* queen and the *vagans* workers was quite peaceful.[2]

The attitude of the *Psithyrus* and the *Bremus* queen toward each other was somewhat different. From the very start the *Psithyrus* paid little or no attention to the latter, but during the first few days the *Bremus* queen avoided her rival whenever they met, and usually turned the tip of her abdomen toward the *Psithyrus,* as if to ward off an attack. However, these signs of hostility on the part of the *Bremus* queen gradually decreased and ceased completely after the fourth or fifth day, and thereafter both queens lived quite peacefully together.

To the *vagans* males, of which several were present when the *Psithyrus* first entered the nest, and to two young *vagans* queens which hatched on September 15, the *Psithyrus* paid no attention whatsoever, nor did any of these young queens and males exhibit the slightest sign of fear toward the *Psithyrus.*

[1] Sladen ('12, p. 253) who observed a *Ps. rupestris* queen, which he had placed in a *B. lapidarius* nest, treat the *lapidarius* workers in a manner similar to that described above, concluded that the workers were too small to get hurt, but, as will be seen later, this is not an adequate explanation.

[2] This agrees with similar observations by Smith ('55, p. 210) and Wheeler ('04, p. 353). According to the former, the *Psithyri* "live on the most friendly terms with the industrious part of the community," and the latter, in the case of ants, found that "the relations between the [*Formica*] *consocians* queen and the *incerta* workers were perfectly cordial."

As in the case of the *affinis-ashtoni* colony, the members of the *vagans-laboriosus* colony became more and more inactive as the weather grew colder. On September 12 the old *vagans* queen disappeared from the nest, and four days later the old *Psithyrus* vanished. The two had lived together for about five weeks.

From what has been said it is evident that *Ps. ashtoni* and *Ps. laboriosus* do not always, if ever, kill the host queen, and in this respect they behave like the two other American *Psithyri* whose hosts are known. One of these, *Ps. insularis,* was found breeding in a colony of *B. flavifrons* by Sladen ('15), and the other, *Ps. variabilis,* was found repeatedly in the nests of *B. pennsylvanicus* by Frison ('16). In the six infested nests reported by these two authors, each one contained the old *Bremus* queen, and this despite the fact that in both cases young *Psithyri* had emerged, or were about to emerge.

All of these observations on the habits of our American *Psithyri* agree with Hoffer's ('81, '88) account[1] and make Sladen's claim (that *Psithyrus rupestris* and *vestalis* always kill the host queen) extremely doubtful. Sladen ('12) based his conclusions on the fact that he never found a living *Bremus* queen in a *Psithyrus*-ridden nest.[2] Against this we have the positive evidence of Hoffer ('88, pp. 126, 148), who, as in the case of *Ps. campestris, quadricolor,* and *barbutellus,* also found the queens of *Ps. rupestris* and *vestalis* living in peace with the host queen, in one case after some of the offspring of the *Psithyrus* had emerged. Further inquiry into the queen-killing habit of *Ps. rupestris* and *vestalis* will undoubtedly show that Sladen ('99, '12, '15) based his conclusions on insufficient evidence.

This same criticism applies to another of Sladen's ('12) conclusions. He (p. 68) believes that *Psithyrus* queens do not kill one another, because he never found a dead *Psithyrus* in a nest ruled by a *Psithyrus.* This certainly is not true of *Ps. laboriosus.* During June, July, and early August, *Ps. laboriosus* queens were very common in the vicinity of Boston, so that numerous experiments along this line could be carried out. The writer repeatedly caught *laboriosus* queens which were searching for *Bremus* nests and placed them in a small box containing comb filled with honey. Whenever two such queens were placed in this box, or in similar receptacles without comb, they immediately engaged in a violent battle which invariably resulted in the death of one of the combatants.

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[1] Recent observations by Wheeler and Taylor ('21) indicate that *Vespa arctica* Rohwer, which has *Psithyrus*-like habits, being a social parasite on *Vespa diabolica* De Saussure, may also sometimes, if not always, live in peace with the host queen.

[2] Such negative evidence, as the work of Wheeler and Taylor ('21) indicates, is very unsatisfactory. Of nine *Vespa diabolica* nests taken by these two authors comparatively early in the season (before August 4), only one contained the old *diabolica* queen, and yet, with a single exception, these nests were not parasitized by *Vespa arctica.*

Psithyrus laboriosus queens also repeatedly appeared in several colonies of *Bremus fervidus* Fabricius and two of *Bremus bimaculatus* Cresson, one consisting of the old queen and about 25 workers, and the other of the old queen, several young queens, and about 50 workers. All of these colonies were kept on the third floor of one of the Bussey buildings. The *fervidus* colonies always expelled these intruders by a unique and very effective method, which will be described in another paper. The two *bimaculatus* colonies, on the other hand, never seriously objected to these intruders, and the latter sometimes stayed in their nests for several days. However, neither of the *bimaculatus* colonies produced any young *Psithyri.*

Psithyrus laboriosus queens which were searching for bumblebee nests were also frequently placed in these *bimaculatus* colonies. If a *laboriosus* queen was already present in the nest, and another one was introduced, the two *Psithyrus* queens usually clenched immediately, and within a minute or two, sometimes within a few seconds, one toppled over, mortally stung. In one or two cases the introduced *Psithyrus* queen tried to avoid a conflict by making a dash for the flight-hole as soon as she noticed the other *Psithyrus.* The two opponents, as a rule, seized each other by one of the legs and endeavored to sting one another. As soon as one had succeeded in penetrating the body of her adversary with her sting, she attempted to extricate herself from the embrace of her vanquished foe. During these encounters it sometimes happened that legs were torn off, or that the dead *Psithyrus* held firmly to one of the legs of the victor with her mandibles so that the latter had to be released. Such a *Psithyrus* in turn was sometimes killed a few minutes later by a third *Psithyrus* which was placed in the nest.

The behavior of the *Psithyrus laboriosus* queens toward the *bimaculatus* workers differed essentially from that described for the *Ps. laboriosus* queen in the *vagans* colony. Every one of the 16 *laboriosus* queens used in these experiments completely ignored the *bimaculatus* queens and workers. Only once (shortly after she had been introduced into the nest) was one of these *laboriosus* queens observed raising one of her middle legs threateningly toward a *bimaculatus* worker, a form of intimidation which is quite frequent, even between members of the same *Bremus* colony.

Psithyrus laboriosus and *Psithyrus ashtoni* queens were also frequently placed in two strong colonies of *Bremus impatiens* Cresson, one having about 125 workers and the other more than 450. As soon as a *Psithyrus* queen was introduced into one of these nests, a great uproar arose in the colony. The workers rushed madly in every direction hunting for the source of the disturbance. The *Psithyrus* queen was seized almost immediately by numerous workers who tried to sting her, and was thus made absolutely helpless. A few bellicose workers, unable to get hold of the *Psithyrus,* seized some of the attached workers instead, and then attempted to sting toward the center of the struggling mass. During one of these experiments (August 15) such a fighting mass was lifted out of the nest box with a pair of forceps. When the workers finally released their hold, it was found that the mass had consisted of 17 workers and the *Psithyrus.* The latter and four of the workers were mortally stung. Although the *Psithyrus* made attempts at stinging during this struggle, one of the four workers was stung to death by one of its fellows at the periphery of the mass, and it is probable that the other three met death in the same manner. At the beginning of two of these experiments two workers, in their excitement, attacked each other (several inches from the *Psithyrus*) and one was stung to death. All of these experiments with the *B. impatiens* colonies always ended with the death of the *Psithyrus.*[1]

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[1] Frison ('21) reports that he found two *Ps. laboriosus* queens, one dead and one paralyzed, in the nests of *Bremus auricomas* Robertson and *B. pennsylvanicus,* and expresses the belief that both were stung to death by the

However, as will be seen from the following incident, a battle between a *Ps. laboriosus* queen and *B. impatiens* workers may have quite a different ending under somewhat different conditions. On July 24, 19 workers of colony No. 8 (*B. impatiens*), which had been transferred to one of the Bussey buildings on the preceding day, were caught at the old nest site and placed in a small glass jar. A few minutes later a *Ps. laboriosus* queen was discovered on some comb which had been left in the empty nest cavity of colony No. 9 (*B. fervidus*). Just to see what would happen, the *Psithyrus* was also placed in the jar. All of the inmates, including the *Psithyrus,* were ill at ease and tried to escape, but one or two of the *impatiens* workers nevertheless attacked the *Psithyrus* queen as soon as they came in contact with her. The latter now went on the warpath herself. She quickly seized one *impatiens* worker after another, whether attacked by them or not, rolled them below her abdomen and stung them to death. This done, she seemed to feel quite at home in the jar and began to lap up the honey which was oozing out from the bodies of her victims. From what has been said before, it is evident that this encounter would have ended quite differently if it had taken place in the nest of the *impatiens* workers. This, as well as some of his own observations (*e.g.,* '12, p. 277), disproves Sladen's ('12, p. 253) claim, already referred to, that the *B. lapidarius* workers, which were mauled by a *Ps. rupestris,* did not get hurt because they were too small.

During the summer of 1905 Wagner ('07, pp. 77, 78) discovered several nests of *Bremus muscorum* Linnæus at some distance above ground, and concluded that the nest-building instinct of this species, which normally builds on the ground, is in a process of transformation. The cause for this change he ascribes to natural selection brought about by the fact that the colonies of this species are destroyed, in large number, by various species of *Psithyrus.* It is improbable, however, that we are here dealing with a change in instinct. Hoffer ('88, p. 95) records that in the spring of 1886

Bremus queens, both of which he found uninjured. However, judging from the non-aggressive attitude of the queens, and the belligerent behavior of the workers of *Bremus impatiens,* the writer would suggest that the two *Psithyrus* queens were stung to death by the *Bremus* workers, which, according to Frison, were present in both nests.

three queens of *B. lapidarius,* which usually builds underground, started their nests in different parts of his house, and that a few weeks later numerous *Ps. rupestris* queens were hunting for these nests. These observations of Hoffer, and the fact that *Ps. laboriosus* queens repeatedly appeared in the writer's *Bremus* colonies kept in a third-story window (about 30 feet above ground), make it evident that *Psithyrus* queens are able to find bumble-bee nests, even if the latter are located at a considerable altitude. Natural selection in this direction is therefore of little benefit to a *Bremus* species as long as the members of the genus *Psithyrus* retain their acute sense of smell.

How exceedingly keen the olfactory sense of these social parasites actually is[1] can be inferred from the following observation: On July 3, an exceptionally pleasant day, colony No. 2 (*B. bimaculatus*) was dug up. The comb had hardly been exposed when a *Ps. laboriosus* queen swooped down upon it. She was captured and a half a minute later another *laboriosus* queen was buzzing about the comb. This one was also caught; but within another minute a third *laboriosus* queen alighted on the comb, and this despite the fact that not a single *Psithyrus* had been noticed in the vicinity previously.

This sudden appearance of the three *Psithyrus* queens also suggests an error in one of Sladen's ('12) experiments. As already stated, this author believes that the *Bremus* workers of a *Psithyrus*-ridden colony are constantly watching for an opportunity to kill the *Psithyrus,* even if the latter has been living with the colony for a considerable period. The observations on which Sladen ('12) chiefly based his conclusion were briefly as follows: On July 9, 1911, this author (cf. p. 251 ff.) dug up a nest of *B. lapidarius* containing 71 workers and a large amount of *Ps. rupestris* brood, but the *rupestris* queen was nowhere to be seen. Suspecting she had hidden herself in a side hole, he left a lump of cocoons in the nest cavity to attract her, and returning a quarter of an hour later actually found a *rupestris* queen on the comb, and concluded that he was dealing with the same *Psithyrus* queen which had been living in the nest. However, judging from Sladen's ('12, p. 253) description of the hostile attitude of this *rupestris* queen toward the *lapidarius* workers, it seems probable that he was not dealing with the mother of the *Psithyrus* brood.

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[1] Observations of Latter ('06) and the Raus ('18) indicate that this is also true of some solitary parasitic bees and wasps.

According to Hoffer ('81, '88), Friese ('88), and Sladen ('12), each European species of *Psithyrus* breeds only in the nests of certain *Bremus* species. Some—*e.g., Ps. rupestris* and *vestalis*—seem to be restricted to a single host, while others, like *Ps. campestris* and *barbutellus,* have two, or even three, hosts. Whether or not our American *Psithyri* have more than one host still remains to be decided. In determining this question we must understand clearly, however, what we mean by the term "host." While it is true that some of the European *Psithyri*—*e.g., Ps. rupestris*—breed only in the nests of a certain *Bremus* species, they may nevertheless be found lodging temporarily in the nests of other species. It will therefore be necessary, if we wish to avoid confusion, to restrict the term "host" to those *Bremus* species in whose nests a given species of *Psithyrus* is known to breed successfully. Using this definition of a *Psithyrus* host as a criterion, we thus far know of only one host for each of the four American species of *Psithyrus* whose hosts have been discovered. They are as follows:

TABLE I.

Psithyrus.	Host.
Psithyrus ashtoni Cresson	*Bremus affinis* Cresson
Psithyrus insularis Smith	*Bremus flavifrons* Cresson
Psithyrus laboriosus Fabricius	*Bremus vagans* Smith
Psithyrus variabilis Cresson	*Bremus pennsylvanicus* De Geer

But, as already pointed out by Sladen ('15), the geographical distribution of *Ps. insularis* and its host, *B. flavifrons*, are not identical, and we must therefore assume that *Ps. insularis* has more than one host, or that *B. flavifrons* also occurs in Saskatchewan, Manitoba, Ontario, Quebec, North Dakota, Minnesota, Wisconsin, Michigan, and New York. This second alternative is rather improbable.

Similar, though less extensive, discrepancies in the geographical distribution also exist in the cases of *Ps. ashtoni* and its host, *B. affinis,* and of *Ps. laboriosus* and its host, *B. vagans*. *Ps. ashtoni* either has more than one host, or *B. affinis* ought to be present on

Prince Edward Island, in New Brunswick, Nova Scotia, Quebec, Manitoba, and Saskatchewan. In this case it is difficult to make any prediction. *B. affinis* occurs in New England, Ontario, and Minnesota, and hence may yet be found in the adjoining eastern and central portions of Canada. This seems all the more probable when we consider that comparatively little collecting has been done in these parts of Canada, and that the number of *Bremus* species known from Illinois has been raised from 10 to 15 during the last few years by the intensive collecting of Frison ('19).

In the case of *Ps. laboriosus* and its host, *B. vagans,* we have to assume either that *Ps. laboriosus* has more than one host or that *B. vagans* is also present in South Carolina and Georgia and on Prince Edward Island. *B. vagans* occurs in Nova Scotia, North Carolina, and Tennessee, and it is therefore probable that it will be reported from the adjoining territories mentioned above.

In the case of *Ps. variabilis* and its host, *B. pennsylvanicus,* the geographical distribution of the former only covers a small portion of that of the latter, and it would therefore not be surprising if *Ps. variabilis* is taken in states from which it has not been reported. In fact, its known geographical distribution makes it almost certain that it occurs in Georgia, Indiana, and South Dakota.

There still remain several other interesting questions in regard to the members of the genus *Psithyrus,* such as origin, similarity in coloration with their hosts, and the frequency with which they breed in *Bremus* nests. The most extensive records which we have as regards the last of these three questions are those for one of the European species, *Ps. campestris.* Hoffer ('88, p. 132) records that he found more than 70 nests of *B. variabilis* and *B. agrorum* victimized by this species. Out of 48 nests of *B. variabilis,* 13 contained *Ps. campestris,* and during several summers in the early 80's about half of the nests of these two *Bremus* species harbored this parasite. In 1910 Frison ('16) opened seven surface nests of *B. pennsylvanicus,* four of which were infested by *Ps. variabilis.* Of the four *B. vagans* nests of which we have records (one each taken by Putnam, Beutenmueller, Franklin, and the writer), only one was later victimized by *Ps. laboriosus,* and of two *B. affinis* nests examined (one by Franklin and one by the writer), one was infested by *Ps. ashtoni,* while *Ps. insularis* was found breeding in the only nest of *B. flavifrons* on record. These figures indicate that in some places, at least in certain years, about half of the colonies of those *Bremus* species which serve as hosts to certain species of *Psithyrus* are parasitized by the latter.

Some of the European *Psithyri—e.g., Ps. quadricolor* and *rupestris*—closely resemble their respective hosts in coloration, while others, like *Ps. campestris,* have no resemblance to their host whatsoever. In the case of the four American species of *Psithyrus* whose hosts are known we have a similar state of affairs. Between *Ps. laboriosus* and its host, *B. vagans,* there is considerable similarity, especially between *Psithyrus* male and host. On the other hand, there is little or no similarity between *Ps. ashtoni* and *insularis* and their respective hosts, *B. affinis* and *flavifrons,* and between *Ps. variabilis* and its host, *B. pennsylvanicus,* there is practically none.

Hoffer ('88, pp. 115, 116) states that those of the European *Psithyri* which are parasitic on *Bremus* species, which are equally large (queen against queen) or larger than they themselves, resemble their hosts more closely than those *Psithyrus* species which prey on smaller *Bremus* species. This correlation in color and size between parasite and host may be true of the European *Psithyri,* but it does not hold for our American species. According to Franklin ('12), the queens of *Ps. ashtoni* and *variabilis* are both considerably smaller than their hosts, *B. affinis* and *pennsylvanicus,* and still, in this case, there is little or no similarity in coloration between parasite and host. On the other hand, the *Ps. laboriosus* queen is somewhat larger than that of its host, *B. vagans,* and here, especially between *Psithyrus* male and host, we have a considerable degree of similarity.

We now come to the very interesting question as to the origin of the members of the genus *Psithyrus.* Müller ('71), Pérez ('83), Hoffer ('88), Sladen ('12), Lutz ('16), and Wheeler ('19), all of whom have paid more or less attention to this question, are agreed that the members of the genus *Psithyrus* are a degenerate offshoot from the genus *Bremus.*[1] One of these authors (Pérez, '83, pp.

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[1] All observations on *Bremus-Psithyrus* colonies indicate that in no case is there a genetic relationship like that suggested by Patterson and Pack-Beresford ('03) for *Vespa austriaca* and *Vespa rufa.* According to this

207–215) believes that the representatives of the genus *Psithyrus,* because of their "perfect homogeneity," could not have come from several *Bremus* species, and, on the basis of structure, tries to show that they have all arisen from a single form which was closely related to *Bremus mastrucatus* Gerstæcker. He believes that in the course of time *Ps. barbutellus, campestris,* and *vestalis* became more differentiated from this original type than the other *Psithyrus* species. Hoffer ('88) and Sladen ('12), on the other hand, believe that there is no evidence for such an assumption, and the last-named author suggests that such resemblances as regards coat-color, etc., between several species of *Psithyrus* and their *Bremus* hosts are "clearly attributable to mimicry or exposure to the same conditions of life and not to ancestry." If we accept this explanation, it is difficult to account for those cases where there is not the slightest similarity between *Psithyrus* and host—*e.g., Ps. campestris* and *Bremus agrorum* Fabricius.

It is no doubt true, as Schmiedeknecht ('07) has pointed out, that in the case of the Bremidæ other criteria, such as length of head, are of greater taxonomic importance than coloration. That the latter is nevertheless of considerable value in determining relationship is indicated by the striking similarity between many of the species of the various English and American *Bremus* and *Psithyrus* groups, as they have been established by Franklin ('12) and Sladen ('12, p. 152), in the one case on the basis of structure, and in the other on the basis of habit (pollen-primers, pollen-storers, and carder-bees).

Using coloration as a basis, the various European and American *Psithyri* can be divided into three groups. The first of these, which may be called the *rupestris* group, is restricted to the Old World. The members of this group strikingly resemble a number of *Bremus* species, likewise restricted to the Old World, with one of which, *B. mastrucatus,* they are very similar in structure, as Pérez ('83) has shown. *Ps. rupestris,* the chief representative of this group, is parasitic on *B. lapidarius,* from which it is difficult to distinguish it.

theory, we should expect the parasitic Hymenoptera to be the ancestors of their hosts, a view which, as Saunders ('03) has pointed out, is difficult to harmonize with certain well-established facts.

Group two, which may be called the *laboriosus* group, and which comprises those *Psithyrus* species which Franklin ('12) has assembled under that name, is restricted to the New World. Here, again, we have a group whose members closely resemble a number of *Bremus* species, also restricted to the New World, one of which, *B. vagans,* serves as host to *Ps. laboriosus,* the chief representative of this group.

The third and largest group, which may be called the *vestalis* group, has representatives in both the Old and the New Worlds. To it belong such European species as *Ps. vestalis, distinctus,* and *quadricolor,* and the members of Franklin's ('12) *ashtoni* and *fernaldæ* groups in America. According to Pérez ('83) and Franklin ('12), many of the representatives of this group are also similar in structure. This group likewise closely resembles a number of *Bremus* species, which in this case are present in both the Old and the New World—*e.g., Bremus occidentalis* Greene in America and *B. latreillellus* and *jonellus* in Europe. The last-named species serves as host of *Ps. quadricolor,* a widely distributed European species belonging to this group.

The great difference in coloration (with a parallel difference in structure) between these three groups, the geographical distribution of those *Bremus* species which resemble them, and the fact that many *Psithyri* are parasitic on similarly colored *Bremus* species, all point to an independent origin of at least three *Psithyrus* groups.[1]

Just how the parasitic habits of the representatives of the genus *Psithyrus* may have originated is suggested by certain observations of Sladen ('99, '12). According to this author, many of the later appearing queens of certain *Bremus* species do not take the trouble to start nests of their own, but enter nests already occupied by their own or other species. In the resulting conflict the foundress of the colony is sometimes killed, and her offspring then assists in rearing the brood of the intruder. He states that this occurs frequently between the queens of *Bremus lucorum* Linnæus and its larger variety, *B. terrestris,* the *lucorum* colony serving as a temporary host. From this temporary social parasitism, as he points out, it is but a step to the permanent social parasitism of the members of the genus *Psithyrus*.[2]

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[1] A similar independent origin has been suggested by Wheeler ('10, p. 449) for certain ants which are temporary social parasites on other ants.

This step in the probable evolution of the permanent social parasitism of *Psithyrus* is found among the ants. As Wheeler ('04, '10, '19) was the first one to point out, there is a large group of ants—*e.g.*, *Formica consocians*—in which the queens are no longer able to found colonies of their own without becoming temporary social parasites on other ants, a form of parasitism which is intermediate between that of *Bremus terrestris* and *Psithyrus*.

Summary and Conclusions.

1. The American *Psithyri*, whose habits have been studied, rarely, if ever, kill the host queen.

2. A *Psithyrus laboriosus* queen which is searching for bumblebee nests never tolerates another such *Ps. laboriosus* queen in the same *Bremus* nest.

3. During the first few days after a *Psithyrus laboriosus* queen has gained admittance to a nest of her host, *Bremus vagans*, the *laboriosus* queen intimidates the workers of the colony by rough treatment. After this period of "getting acquainted," the members of the *vagans-laboriosus* colony show no more hostility toward each other than the members of an uninfested *vagans* colony.

4. The *Psithyrus laboriosus* queen does not always, if ever, lay her eggs in a pollen mass in which *Bremus* eggs, or larvæ, are present, but, like the *Bremus* queen, constructs her own egg-cells and, like the latter, attaches them to one or more cocoons.

5. In *Psithyrus*-infested nests of *Bremus affinis* and *Bremus vagans* the destruction of the eggs, or young larvæ, of the host is probably caused by the *Psithyrus*, and not by her offspring.

6. It is not necessary for the *Psithyrus ashtoni* queen, and probably also not for other *Psithyrus* queens, to forage for her offspring in order that the latter may thrive.

7. The representatives of the genus *Psithyrus* have probably originated from several species of *Bremus* rather than from a single one.

8. Similarity in coloration between *Psithyrus* and *Bremus* spe-

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[2] Wheeler ('10, p. 451) considers it probable that the permanently parasitic ants went through a similar evolution.

cies is probably due to genetic affinity, and not to "mimicry or exposure to the same conditions."

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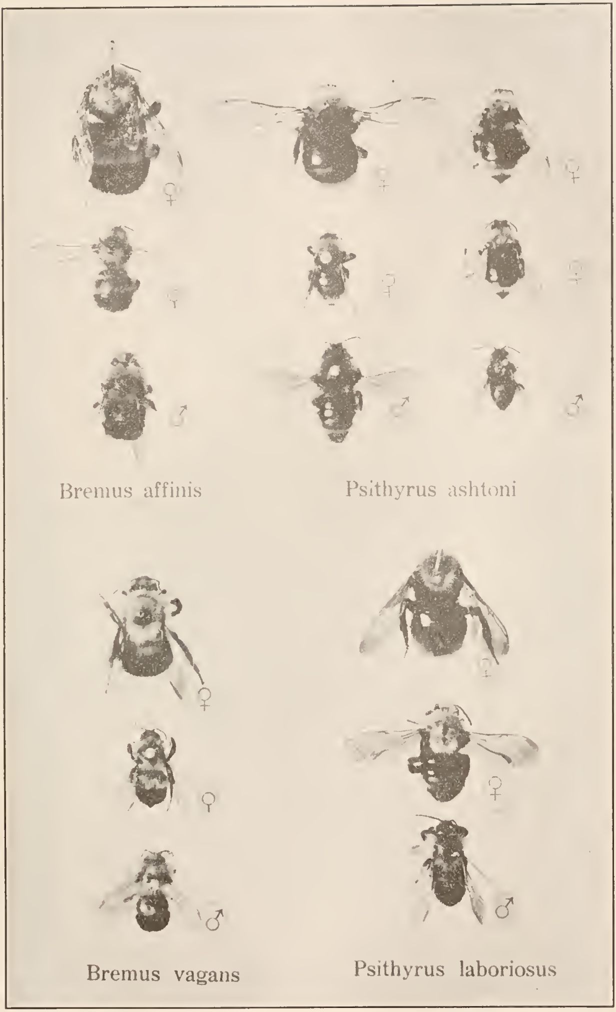
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THE MULTIPLE TESTIS IN URODELES.

R. R. HUMPHREY,

Department of Histology and Embryology, Cornell University,
Ithaca, New York.

In a recent paper ("The Interstitial Cells of the Urodele Testis," 1921) the writer referred briefly to the occurrence, in various urodeles, of what might be termed a multiple testis—a testis made up of a series of enlargements, each of which is morphologically and functionally similar to the others or to the simple testis found in the majority of American urodeles. Successive enlargements are separated by constricted regions, often of greater length than the enlargements themselves. Such regions have been referred to by investigators as "sterile." This designation, however, is clearly inapplicable, since, as we shall see, reproductive cells are not absent.

Of the twelve species of American urodeles examined by the writer, multiple testes are found to occur in three—*Desmognathus fusca, Diemyctylus viridescens,* and *Diemyctylus torosus.*[1] Among the urodeles of Europe, according to Champy, axolotl and the tritons exhibit this feature. I myself have examined testes of the European *Salamandra atra* and find them to consist of as many as three enlargements or lobes.[2]

Though the multiple testis is of rather common occurrence, no satisfactory discussion of its origin and significance has yet been encountered in the literature. Following are a few of the most

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[1] von Wittich ('53) describes and illustrates (his Fig. 18) the testis of *Necturus lateralis* (*Necturus maculosa*) as consisting of three parts or lobes. Hoffmann ('73–'78) in Bronn's "Klassen und Ordnungen der Amphibien", states that von Wittich also describes the testis of *Menopomă* (*Cryptobranchus allegheniensis*) as of the multiple type. The author has found in *Necturus* no testes such as that illustrated by von Wittich, though about sixty males have been examined; neither has a multiple testis been found in any of the six *Cryptobranchus* males studied.

[2] Each enlargement or secondary testis will in this discussion be termed a lobe, a designation agreeing with that employed by Champy and other workers.

pertinent comments: Kingsbury ('02) noted the occurrence of lobes in the testis of *Desmognathus*. He says: "There seems to be no absolute correlation of this condition with other structural features of the salamander, save that the presence of two or more enlargements occurs more often—in fact, quite constantly—in large animals. A similar division of the testis into 'lobes' occurs in other salamanders with an elongated body, and has been noted in *Amphiuma* and *Spelerpes*.[1] The segmented condition of the organ in *Cœcilians*[2] is perhaps to be associated likewise with the elongated form of the body."

Champy, similarly, comments on the relation of body size to the presence of the multiple type of testis. He believes that the variation in the number of lobes is correlated with that in the size of the species. He found the lobes very numerous in axolotl, more numerous in *Triton cristatus* than in *Triton palmatus* and *Triton punctatus,* and least numerous in the salamanders, though the last-named animals, he concedes, are of rather large size. Champy further considers that among the individuals of any species the number of lobes will be proportional to the size of the animal. He states that animals reared with insufficient food remain small and their testes develop few lobes, while an animal of one year, well nourished, may possess a testis of numerous lobes.

From my own observations on the testes of numerous species of American urodeles it would appear that the general conclusions stated by previous workers are in part inapplicable. The size of the species appears to be in no way correlated with the occurrence of a multiple testis. For example, our largest species, *Cryptobranchus* and *Necturus,* have testes of the simple type; three species of medium size—*Plethodon glutinosus, Gyrinophilus porphyriticus,* and *Amblystoma punctatum*—likewise have testes of unit structure. The species possessing the multiple testes (*Desmognathus* and *Diemyctylus*) are, in comparison with the above, small animals, yet their testes may consist of from two to five lobes. Plainly the size of the species is not to be regarded as a factor.

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[1] The writer is unable to confirm the occurrence of a multiple testis in *Spelerpes*, though about a dozen males of the species *Spelerpes bislineatus* have been examined. McGregor ('99) likewise states that no division of the testis into lobes occurs in *Amphiuma.*

[2] The multiple testes of the Cœcilians, judging from the descriptions available, are probably of a type different from those of the *Urodeles*. Spengel ('76) describes the enlargements as being numerous in an immature animal, and states that they are separated by regions from which germ cells are entirely absent. Urodele males show no such primary growth centers; neither are germ cells entirely absent from any region of the testis.

That the elongated form of the body is responsible for the multiple testis is likewise hardly conceivable. Among our American urodeles none are more slender and graceful of body than *Gyrinophilus* and *Spelerpes bislineatus.* Yet in these animals the testis, though an elongated structure, is not divided into lobes. *Desmognathus* and *Diemyctylus,* possessing multiple testes, are comparatively stout-bodied animals.

Champy's conclusion that the number of lobes in males of the same species is proportional to their body length is only in a limited sense correct, as the following figures from my records show: The average length of eight *Desmognathus* males with testes of two lobes each is 9.8 cm.; the average length of six males with testes of three lobes is 10.2 cm. or 0.4 cm. greater. Nevertheless one of the individuals of the first group measured 12.2 cm., while no animal of the second group exceeded a length of 10.5 cm. The average body length for the same group of eight males having testes of two lobes each is 9.8 cm. Within this group, however, are lengths ranging from 8 cm. to 12.2 cm. Clearly other factors than mere body length must be concerned, else we should find much less variation within this group.

Conceding that males which have been kept small by lack of food may develop testes of few lobes, as Champy states, the writer is forced, so far as *Desmognathus* is concerned, to doubt Champy's further claim that animals well fed may become very large, and, at one year of age, possess testes of numerous lobes. Since the urodele male completes but one spermatogenetic cycle annually, the males of one year referred to by Champy would of necessity reach sexual maturity with testes of many lobes already developed. In other words, the multiple testis, whatever its correlations, would develop simply through the initiation of greater growth activity in localized regions of the original cord of germ cells, as suggested by Kingsbury ('02). A male approaching sexual maturity might, on the basis of this theory, possess an even greater number of lobes than one which had been sexually active for several seasons.

Champy, indeed, states his belief that the number of lobes is not at all dependent upon the age of the male.[1] In *Desmognathus,* however, the evidence shows clearly that the sexually immature male, regardless of size and vigor, possesses only a simple testis—*i.e.,* only one lobe is present. The multiple testis, then, must arise by the addition of other lobes through growth processes *following sexual maturity;* in other words, the *age* of the animal must be considered as a factor.

Preceding investigators, so far as can be ascertained, have merely noted the occurrence of the multiple testis and speculated as to its possible correlations. The origin of the numerous enlargements or lobes has not been adequately investigated, and it is to their origin and development that we must turn if we are to fully appreciate their significance. Spengel ('76) makes the brief statement that the numerous lobes of a multiple testis arise as the result of the complex growth, degeneration, and regeneration processes of the organ.[2] This statement, properly elaborated, furnishes, I believe, the correct interpretation of these structures. The pattern of the spermatogenetic processes, in other words, explains the multiple testis. It is clearly not a structure of segmental origin; its correlation with the body size or body form of the species or the individual of the species can not be established. It is, as I shall attempt to show by a discussion of the urodele plan of spermatogenesis, a structure arising from the combined operation of three factors: (1) the slow movement of the spermatogenetic "wave"; (2) the delayed regeneration of the emptied lobules at the close of the spermatogenetic cycle; (3) the age of the animal—*i.e.,* the number of sexual cycles through which the two first-named factors have been operative.

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[1] An earlier French worker, Duvernoy ('51), according to Spengel, reached a similar conclusion. Spengel (p. 65) says: "Er glaubt, die Zahl der Abschnitte sei allein abhängig von der Brunst, da er keine constanten Altersunterschiede zu entdecken vermochte."

[2] Spengel (p. 65) says: "Die eingehendere Erörterung dieser Frage muss ich bis zur Darstellung der Entwickelung und des Wachsthums verschieben . . ." Whether Spengel ever published a more detailed explanation of the multiple testis, as the foregoing quotation shows he proposed doing, I am unable to state. No reference to any later work by him on the amphibian testis has been encountered in the literature, although his first publication, "Der anatomische Bau des Urogenitalsystems," is often extensively quoted.

The structure of the urodele testis has been described at length in a previous communication and need be only briefly reviewed at this time. The elongated testis is suspended from the dorsal abdominal wall by a mesorchium in which run the blood vessels and efferent ductules. The latter, varying from few to several in number, lead from the longitudinal collecting duct of the testis to the ductus deferens (Wolffian duct). The longitudinal collecting duct may be superficial in position, or as in many urodeles, more centrally located. The structural units of the testis, the lobules,[1] empty into the longitudinal collecting duct either directly, by means of very short ducts, or more indirectly, by way of longer, much-branched tributary ducts.

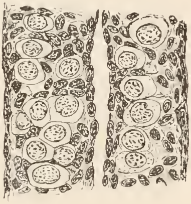

Fig. 1. Longitudinal section of the extreme anterior end (primary germ-cell cord) of a *Desmognathus* testis. The longitudinal collecting duct is shown, bordered by primary spermatogonia. The caudal germ-cell cord remaining after the complete degeneration of the lobules is essentially the same structure, as is also the slender cord intervening between successive lobes. The primary spermatogonia, in these latter structures, are properly termed "residual" spermatogonia.

A study of the lobule itself shows it to be a structure made up of reproductive cells all in approximately the same stage of development. Figure 2 shows the lobules as hollow cyst-like chambers lined by spermatogonia. All the cells in a lobule undergo their developmental transformations synchronously, mature as spermatozoa and leave the lobule. There remain behind only the Sertoli cells—which soon degenerate and disappear—and the residual spermatogonia. The latter are germ cells located only in the apex of the lobule, where it joins the collecting duct system.

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[1] The term lobule, introduced by Kingsbury ('02), is used here in preference to the terms "cyst," "capsule," "tubule," etc., employed by various investigators.

Though present, they have remained quiescent during the months preceding the extrusion of the spermatozoa; with the emptying of the lobule, however, or even somewhat previously in many urodeles, they begin to multiply. By increase in their numbers the emptied lobule is in time completely regenerated; its germ cells again come to maturity and the cycle of degeneration and regeneration is repeated. That a delay in the regeneration of a group of emptied lobules would appreciably modify the form of the testis is self-evident. Such delayed lobule regeneration, as a factor in the formation of multiple testes, will be referred to subsequently.

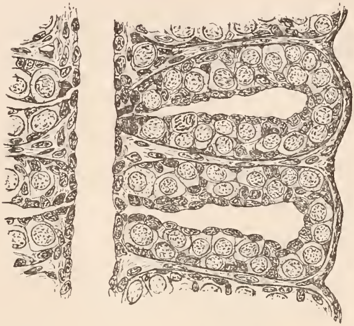

FIG. 2. Longitudinal section of a *Desmognathus* testis, showing a region somewhat posterior to that pictured in Fig. 1. Developing lobules are here well defined and their relation to the collecting duct clearly indicated.

All the lobules of the urodele testis do not, as a rule, mature and discharge spermatozoa at the same time. Examination of the testis shows the older developmental stages of the germ cells to be localized in its caudal region, while the earlier or younger stages are found more cephalically. In the course of spermatogenesis a wave of developmental change passes from the caudal to the cephalic end of the organ. The lobules of any region, therefore, will contain reproductive elements similarly advanced in development; caudal to any such region older stages are encountered while anterior to it are successively younger stages. Finally, at the anterior end of the testis will be found a slender cord or filament (see Fig. 1) containing only primary spermatogonia.

The caudocephalic movement of the spermatogenetic processes, or, as it has been appropriately termed, the spermatogenetic wave, varies greatly in rate with the species of urodele considered. In *Amblystoma* and *Cryptobranchus* it is comparatively rapid. As a result all regions of the testis tend to be in approximately the same stage of development at any particular time. In other urodeles, such as *Necturus* or *Gyrinophilus,* the rate is considerably slower, and in these several developmental stages will usually be encountered in cephalocaudal succession. Finally, in *Desmognathus* and *Diemyctylus* the rate is extremely slow, the spermatogenetic wave traversing only a part of the length of the testis in any one sexual cycle.[1] At a certain period in the cycle the secondary spermatogonia toward the anterior end of the organ cease to transform into spermatocytes I. A few lobules of these spermatogonia may degenerate entirely or in part, as described by Kingsbury and Hirsh ('12). These degenerated lobules mark a "boundary plane" caudal to which, in the current season, all the reproductive cells are matured as spermatozoa. Anterior to this boundary plane the germ cells are held over until the following season, when the wave resumes its forward movement from the point at which development was checked in the preceding season.

The testis of *Desmognathus,* then, is characterized by a slow movement of the spermatogenetic wave. Consider, now, the action of this factor, in connection with delayed lobule regeneration, as to possible effect on the structure of the testis. The testis of *Plethodon,* which is always of unit structure, may be used for comparison with that of *Desmognathus,* since the two agree in possessing a central[2] longitudinal collecting duct round which the lobules are arranged as are the spokes of a wheel. The two are in contrast, however, both as to the rate of the spermatogenetic wave and the rapidity of lobule regeneration, after extrusion of the spermatozoa. In *Desmognathus* both processes go on slowly; in *Plethodon* both proceed with comparative rapidity, especially the processes of lobule regeneration.

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[1] The urodele male, it will be recalled, completes but one sexual cycle annually.

[2] The collecting duct in *Desmognathus* may, however, be more or less superficial towards the ends of the enlargement or lobe.

In developing males of either *Desmognathus* or *Plethodon,* the testis, subsequent to the establishment of the urino-genital connection, consists of but a slender cord of primary spermatogonia much as in Fig. 1, along whose entire length extends the longitudinal collecting duct. So far the testes of the two animals are substantially alike. Neither shows any indication of the establishment of growth centers such as might result in the formation of a many-lobed organ. Preceding the onset of sexual maturity the spermatogonia of the caudal end of the germ cell cord begin division, and eventually fully developed lobules are formed. This development, of course, proceeds caudocephalically in both animals. In *Plethodon,* whose wave rate is the more rapid, lobules are developed and spermatozoa are matured, in the male's first year of sexual maturity, throughout a considerable part of the length of the original germ cell cord. In *Desmognathus,* because of the slower wave rate, spermatogenesis is checked after proceeding but a fraction of the length of the gonad. A boundary plane is established in the manner previously mentioned, and spermatozoa are matured only caudal to its location.

At the time of maturity of the first spermatozoa, then, the testes of the two animals agree in consisting of but one lobe each. That of *Plethodon* is represented diagrammatically by Fig. 24 of Chart I., in which the unshaded area represents the part maturing spermatozoa. The testis of the young *Desmognathus* male is represented by Fig. 1, in which the same scheme of shading is employed. The boundary plane is indicated by the line *b*. The smaller fraction of the testis becoming functional in *Desmognathus* is to be noted.

Following the extrusion of the spermatozoa, in *Plethodon,* lobule regeneration proceeds rapidly. (It begins, indeed, considerably before the spermatozoa leave the lobule.) Though the form of the organ, before all of the more anterior lobules have been emptied and regenerated, will appear as in Fig. 25, prompt regeneration of the lobules during the winter—the spermatozoa being extruded in autumn—brings the testis, by spring, back to the type shown in Fig. 24. The process is repeated in succeeding seasons with no essential variation, save that the spermatogenetic wave, as the male becomes older, travels each year over a greater extent of the anterior portion of the germ cell cord (represented in black in Fig. 24) in which, during the first season of the male's sexual activity, the germ cells did not develop beyond the secondary spermatogonial stage. In this way the testis is increased in length somewhat from year to year as the animal develops.

In the *Desmognathus* male, however, the extrusion of the spermatozoa in autumn is *not* followed, as in *Plethodon*, by prompt regeneration of the emptied lobules. The few residual spermatogonia located at the apex of each lobule appear to remain quiescent for several months. During the winter the emptied lobules slowly degenerate. The connective tissue cells surrounding them hypertrophy, forming interstitial cells, as described in detail in a previous communication. In the following summer the testis appears as in Fig. 2 of Chart I. The region anterior to the boundary plane *b* in Fig. 1 has developed to form the functional testis, in which the forward movement of the spermatogenetic wave proceeds slowly until again checked at the new boundary plane *b′*. The region containing degenerating lobules and interstitial cells is shown posterior to *b* (cross-hatched). It is to be kept in mind, of course, that the longitudinal collecting duct, surrounded by scattered groups of residual spermatogonia, extends throughout this region. The degenerating lobules and interstitial cells gradually disappear, until finally only the collecting duct and residual spermatogonia remain. In Fig. 2*A* the testis is represented as seen late in the summer following the animal's first year of sexual activity. The extreme caudal part, reduced to a condition similar to that of the anterior cord of spermatogonia, may be referred to as the "caudal germ-cell cord". The functioning region of the testis shown in Fig. 2*A* empties, and the cycle of changes outlined above is repeated. The caudal germ-cell cord, in this way, is increased in length, as shown in Fig. 3. This figure represents the testis of a male as in the summer after the second extrusion of spermatozoa. The positions of the boundary planes of successive seasons are indicated by *b, b′*, and *b″*.

Finally, after several months of inactivity, the residual spermatogonia in the most posterior part of the caudal germ-cell cord begin to multiply. Their multiplication leads to the formation of lobules of secondary spermatogonia; these in due season become spermatocytes I. and finally mature as spermatozoa. The testis,

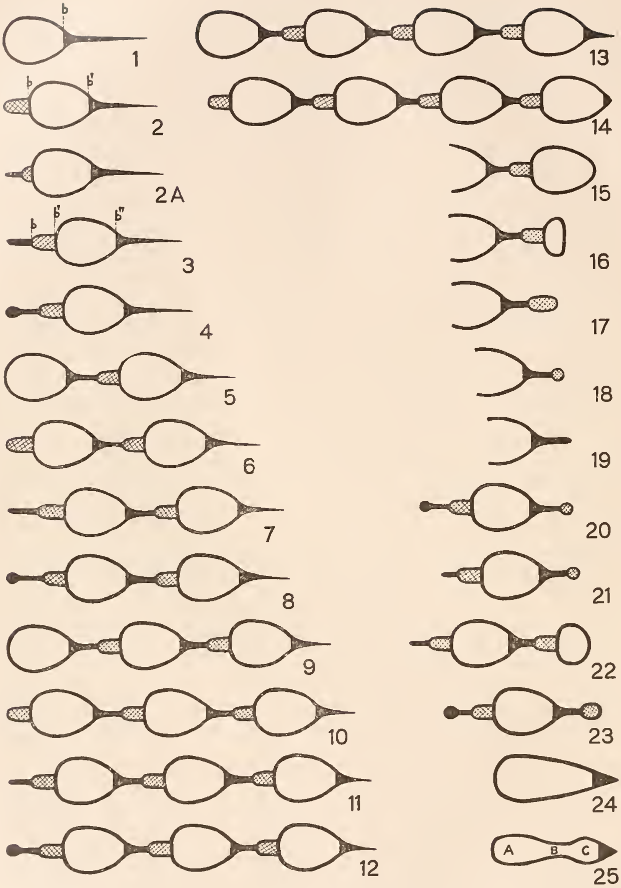
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Chart 1. Figs. 1 to 14. Diagrams illustrating the origin and development of the multiple testis of *Desmognathus,* up to a four-lobed stage. See Tables I. and II. for average lengths of animals having testes of these types. The cephalic end of the testis is at the right in all these figures. Solid black represents regions occupied by primary or secondary spermatogonia. Unshaded areas are those occupied by later stages of the germ cells—spermatocytes I. to mature spermatozoa. Cross-hatched areas are at the time the lobules first begin regeneration, appears as in Fig. 4. A completely developed second lobe is represented in Fig. 5.

The spermatogenetic wave moves slowly cephalad along the caudal germ-cell cord in the same fashion as it moved along the primary germ-cell cord in the case of the first lobe. Boundary planes are established, in each season, in the same manner; anterior to these the transformation of the spermatogonia into spermatocytes I. ceases. In other words, a second "testis" has been established, caudal to the first and separated from it by the so-called sterile region—a region sterile only in the sense that its germ cells are temporarily inactive. This "testis" or lobe shifts forward on the trail of the first, leaving behind it, when first it empties, a caudal degenerating region such as is represented in Fig. 6. By the disappearance of the degenerating lobules and interstitial cells,

occupied by degenerating lobules and interstitial cells. Fig. *2A* represents the testis as seen in later summer; all other diagrams are as of testes in June after the boundary plane (*b*, *b'*, etc.) for the season's development of spermatocytes has been established.

FIGS. 15 TO 19. Diagrams of the cephalic portion of the testis of *Desmognathus*, illustrating the disappearance by "running out" of an anterior lobe. Regions of the testis are represented as in the preceding figures. Figs. 15 and 16, testes as in June. Fig. 17, the same testis as in Fig. 15 or 16, but in June one year later. Fig. 18, the same testis later in the summer, prior to the final disappearance of the lobe. Fig. 19, the same testis in September after the last trace of the lobe has disappeared.

FIGS. 20 AND 21. Diagrams of multiple testes of *Desmognathus* approaching a simple type by reduction of the number of lobes.

FIGS. 22 AND 23. Diagrams indicating the differences observed between the testes of an individual. One testis (Fig. 23) is a season ahead of the other in development, as indicated by the earlier disappearance of the anterior lobe and the earlier development of a new lobe caudally.

FIG. 24. Testis of *Plethodon* male for comparison with Fig. 1. Represented as in midsummer. Note the absence of a long anterior germ-cell cord.

FIG. 25. The testis of Fig. 24 as in the fall, some time after extrusion of the spermatozoa had begun. Region *A* contains developing lobules of secondary spermatogonia for the next sexual cycle. Region *B* contains less well-developed lobules, and many degenerating lobules and interstitial cells. In region *C* the lobules still contain spermatozoa, but the lobules of the next cycle are already beginning their development. Black in this and in Fig. 24 indicates the part of the testis not maturing spermatozoa in the first season; *i.e.*, a region containing only primary and secondary spermatogonia. Fig. 25 illustrates the nearest approach to lobation in the testis of *Plethodon*; the rapid development of lobules in regions *B* and *C* soon restores the testis to the type of Fig. 24.

from this region, a caudal germ-cell cord is again established as in Fig. 7; at the posterior end of this cord, eventually, lobule regeneration begins and a third lobe (see Figs. 8 and 9) is produced in exactly the same way as was the second. In this way multiple testes of as many as five lobes may come into being, though this number, I may add, has been found in but one specimen. Likewise, but one specimen with testes of four well-developed lobes has been noted.

The exact time interval that elapses between the first extrusion of spermatozoa and the regeneration of the region they occupied as a caudal second lobe has not been stated in the above description. As lobules emptied in autumn degenerate very slowly, and the interstitial cells marking their former location persist even until the following autumn, the time interval before the emptied region is completely reduced to a simple germ-cell cord is approximately a year. The spermatogonia in this cord, it appears, do not begin their development at any fixed time after the final disappearance of the degenerated lobules, but may remain quiescent for several months longer. Testes similar to those in Figs. 3 and 4 have been taken from specimens killed in June. Judging from the length of the caudal germ-cell cord as compared with that of the functioning testis, the formation of the second lobe could not be considered as having taken place in less than sixteen to eighteen months after the region first matured germ cells. Since the tiny caudal lobes found in June or July will contain only spermatogonia during the current season, they will not ripen spermatozoa until the end of the following summer. In short, the second lobe of a multiple testis may possibly come to full functional maturity only after an interval of three years from the time its territory of the testis first matured sex cells—that is, in the animal's third season of sexual activity. Since after the disappearance of the degenerating lobules and interstitial cells there is no means of distinguishing regions in the caudal germ-cell cord, it is doubtful whether the exact time interval before lobule regeneration begins can be determined. It would appear, indeed, that this time varies in different animals, since in males killed at the same time, with testes having small second lobes developing, these lobes will vary considerably in degree of development; though, doubtless, they are all to be referred to regions of the testis emptying in the same autumn, in some animals the regeneration of the lobules has been more delayed than in others. It is doubtful, too, whether a caudal lobe, when it develops, occupies exactly the same extent of the germ-cell cord as was occupied by the lobe which preceded it. Caudal lobes of animals killed in September or October may show only a half dozen lobules of mature spermatozoa, or they may be, on the other hand, almost or quite equal in size to the lobes anterior to them. The balance of metabolic processes in the animal doubtless determines the extent of movement of the spermatogenetic wave in any season; hence its cephalic progress in a caudally developing lobe may be checked and the size of the lobe limited quite independently of the exact time that had elapsed since the region was first emptied. The caudal lobe of Fig. 5, therefore, is not necessarily the exact equivalent, in point of territory occupied, of the testis of Fig. 1, but may occupy a greater or less extent of the germ-cell cord.

In each year of sexual activity, as has been stated, the most anterior lobe of the testis is shifted forward by exactly the extent of the region maturing and extruding spermatozoa. It is inevitable that eventually this anterior lobe will reach the cephalic end of the anterior germ-cell cord. By the time this occurs, however, there will be one or more functional lobes established posterior to it. Hence the animal's sexual activity is in no way interfered with by the final disappearance of an anterior lobe.

Such a disappearance or " running out " may be as easily demonstrated as is the caudal addition of new lobes. In Fig. 15 is shown an anterior lobe cephalad of which there is no cord of primary or secondary spermatogonia. This condition marks the first stage in the disappearance of the lobe. In Fig. 16 is represented a lobe somewhat similar to the above, save that it is much shorter; evidently, in the preceding year, only a comparatively small number of lobules of secondary spermatogonia had remained ahead of the boundary plane when it was established, and had been held over for later development. In Fig. 17 a more advanced stage in the running out of a lobe is represented. Here the most anterior lobules of the germ-cell cord have matured and extruded spermatozoa, and are found undergoing degenerative changes, surrounded by sheaths of interstitial cells. Testes of the type shown in Figs. 15 and 16 are frequently encountered in June or July. In another year the testis of Fig. 15 would reach the stage shown in Fig. 17. Then, by the disappearance of the degenerating lobules and interstitial cells during the summer and early fall, all trace of the lobe finally vanishes, as illustrated in Figs. 18 and 19, and only a slender germ-cell cord remains.

The number of lobes that may possibly be formed, then, is limited by the extent of the primary germ-cell cord. This may and doubtless does increase by growth in length of its anterior portion as the animal develops. In any event, it varies considerably in different males, since the anterior lobe may appear as in Fig. 16 or 17 even when only two lobes are present, or it may show no indication of running out in a testis of from three to four lobes. An unusually long germ-cell cord permits the development of numerous lobes; a short cord reduces the number, though in no case has it been apparent that a very large old *Desmognathus* male possessed but a single lobe. Animals have been found, however, with multiple testes (as in Fig. 20) consisting of one large functional lobe, a very small lobe developing caudally, and a trace of an anterior lobe. Similar to these, but one step nearer to a simple testis, is that represented in Fig. 21. In this case no caudal lobe has yet begun its growth, and the anterior lobe is reduced almost to total disappearance. This type of testis in *Desmognathus* is exceptional, only one having been encountered in an examination of over a hundred males.

From the preceding description it is readily seen that a combination of two factors is essential in the production of the multiple testis of *Desmognathus*. These two factors, let me repeat, are the slow movement of the spermatogenetic wave and the delayed regeneration of the emptied lobules. Neither of them alone could give rise to a multiple testis. If, for example, the slow spermatogenetic wave is combined with rapid regeneration of the lobules, the length of the testis is merely increased each year by additions from the anterior germ-cell cord, but remains a unit, as in the *Plethodons*. If, on the other hand, a rapid spermatogenetic wave passed the complete length of the testis in a single season, as in *Cryptobranchus,* but lobule regeneration were then delayed for the length of time that it is in *Desmognathus,* the animal would, of necessity, pass an interval of one or more years of sexual inactivity before reproduction again became possible. Such a condition, of course, does not exist in any known urodele.

Even in an animal possessing the spermatogenetic pattern requisite for the production of a multiple testis it is further essential that considerable time elapse after sexual maturity before a second testicular lobe is developed, and, following this, still another interval before the appearance of a third. All the evidence from *Desmognathus* is contradictory to Champy's assertion that age is not a factor, and that a male one year of age may possess testes of several lobes. Sexually immature males with more than one lobe are unknown to the writer. Large, heavy-bodied males invariably have multiple testes; these males, however, are always sexually mature, as shown either by the presence of spermatozoa or the occurrence in the testis of degenerating lobules emptied in a previous sexual cycle. Further, these large, heavy-bodied males, when one takes them in large numbers from their natural environment, must also average *older* than slender-bodied males of half to two thirds their body length taken from the same environment. Males of this last class average a smaller number of testicular lobes. *In short, the younger the animal, the smaller the number of lobes that will be found to have developed.*

As previously stated, the writer does not consider that the number of lobes is associated with body size alone, as claimed by Champy. Since, however, body size is to a certain extent correlated with age, an incidental correlation between lobe number and body length may be expected as a result. Examination of *Desmognathus* males shows some sexually mature animals to be only of 6 to 7 cm. total length, while the largest males measure 10 to 12 cm. A graphic representation of the relation of size to the form of the testis is presented in chart II. For graph *A,* stages 1 to 14 in the development of a multiple testis, taken from chart I., are used as abscissæ, and the corresponding average body lengths of the males, as given in Table I., are used as ordinates. It will be noted that up to stage 5 there is a fairly gradual increase in body length, along with the increase in the number of lobes. Animals at this stage—

TABLE I.

Lengths of Desmognathus Males from Streams on Turkey Hill, near Ithaca.

Arranged in groups on the basis of the type of testis. These types, listed in first column, correspond in number with the types illustrated in chart I. The one animal with testis of five lobes is not included in this table; since it had suffered the loss of a portion of its tail, its length could only be estimated.

Testis Type No.	Number of Lobes.	Number of Specimens Examined.	Length in Centimeters.		
			Maximum in Group.	Minimum in Group.	Average of Group.
1	1	4	5.8	5.0	5.4 −
2	1 +	11	8.0	5.5	6.7 +
3	1 +	10	9.5	7.3	8.3 −
4	2	18	10.2	8.0	8.6 +
5	2	8	12.2	8.0	9.8 −
6	2 +	16	12.2	8.0	9.8 +
7	2 +	6	11.2	8.0	10.2 −
8	3	5	10.7	10.0	10.3
9	3	4	12.0	9.0	10.6 −
10	3 +	6	10.5	9.7	10.2 −
11	3 +	0	—	—	—
12	4	0	—	—	—
13	4	1	11.0	11.0	11.0
14	4 +	2	12.0	10.5	11.2 +

that is, with testes of two complete lobes—have reached practically their full size, since after this, if we disregard the three animals representing stages 13 and 14, there is but slight upward or downword shifting in the level of the curve. Hence the maximal size may be attained when the testis is at stages 5 to 9, but the number of lobes may continue to increase for some time before checked by the running out of the anterior lobe at the cephalic end of the germ-cell cord. If the correlation were merely one of size rather than of age, one would expect a curve of quite a different type—a curve continuing upward in the same direction as the portion 1 to 5. This portion of the curve, incidentally, shows the correlation of size and lobe number in the sexually mature but growing male; after full size is attained lobe number still increases, and the last half of the curve maintains a rather constant level.

Graph *A* is based upon animals taken largely from streams on Turkey Hill, about three miles from Ithaca. The animals of these

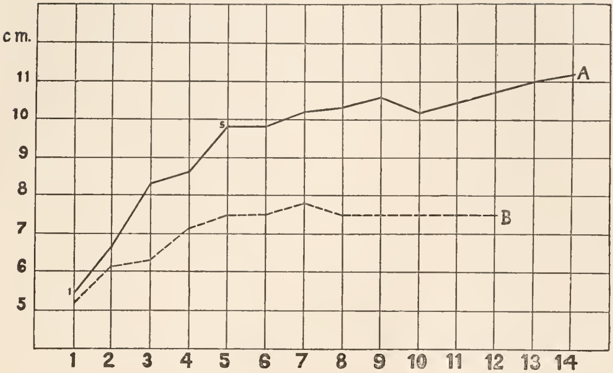

CHART 2. Graph *A* illustrates the relation of testis structure to size in the 93 *Desmognathus* males listed in Table I. The structural types from the first column of this table (see chart 1, Figs. 1 to 14) are here used as abscissæ, and the corresponding average lengths from the last column are used as ordinates. Between stages 5 and 14 an increase in length of but 1.2 cm. is indicated; the number of lobes of the testis increases with age, while the body size, after a certain period, remains relatively fixed. Between stages 1 and 5, on the other hand, there is an increase in length of 4.4 cm. Males mature spermatozoa long before they have attained their maximal size; the processes that operate to produce a multiple testis begin at sexual maturity; hence the development of the first additional lobe is accompanied by the continued increase in body length.

Graph *B*, constructed in the same manner as Graph *A*, shows the relation of testis structure to size in the 24 animals listed in Table II. Between stages 5 and 12 there is here a variation of only 0.3 cm. in average length, as compared with the increase of 1.4 cm. between stages 2 and 5. These animals average much smaller than the animals of the first group, as may be readily seen from the relative heights of the curves at any point. Running out of anterior lobes occurs relatively early and comparatively few lobes are developed; note that stages 9 to 14, represented by 13 males in Table I., have but one representative in Table II.

streams are the largest found in the vicinity of Ithaca, and are uniformly of a lightly colored type. Animals taken from the various gorges nearer Ithaca are ordinarily of smaller size and darker coloration. These have been grouped separately and a second graph (*B*) constructed which agrees in general with the first. For data concerning these animals, see Table II. The animals in this group, as will be seen, are of much smaller average size and do not develop as many lobes as do the animals of the first group.

The multiple testis being purely the expression of the spermatogenetic wave rate and the time required for lobule regeneration, it follows that conditions modifying these factors will through them modify the form of the testis. Favorable metabolic conditions may increase to some extent the wave rate. The wave will travel farther in a season—*i.e.*, a greater number of lobules of secondary spermatogonia will develop into spermatocytes I. before such development is checked for the season and a boundary plane established. As a result the functional lobe will be of greater length than had the wave rate been slower. Lobes of large vigorous males, it is found, tend to be longer than those of smaller specimens. Greater lobe length, in turn, acts to reduce the number of lobes that may develop from a germ-cell cord of given length. Increase of the spermatogenetic wave rate beyond a certain limit, clearly, would reduce the number of lobes to one, and a multiple testis would not be developed.

Unfavorable metabolic conditions, similarly, may still further reduce the normally slow wave rate of *Desmognathus*. This would

TABLE II.

Lengths of Desmognathus Males of Small Dark Type, taken from Gorges near Ithaca (Largely from Fall Creek Gorge on Cornell University Campus.)

Arranged in groups on the basis of the type of testis. The type numbers in the first column correspond to the type numbers of the diagrams of chart I.

Testis Type No.	Number of Lobes.	Number of Specimens Examined.	Length in Centimeters.		
			Maximum in Group.	Minimum in Group.	Average of Group-
1	1	1	5.2	5.2	5.2
2	1 +	1	6.1	6.1	6.1
3	1 +	5	6.9	5.5	6.3
4	2	5	8.1	6.1	7.1
5	2	2	7.2	7.7	7.5
6	2 +	5	8.2	6.0	7.5
7	2 +	3	8.0	7.5	7.8
8	3	2	8.0	7.0	7.5
9, 10, 11	3 +	0	—	—	—
12	4	1	7.5	7.5	7.5
13, 14	4 and 4 +	0	—	—	—

result in the formation of shorter lobes and permit the establishment of a greater number before a cephalic running out of the first lobe occurred. It seems probable that the presence of several lobes is of itself a factor in reducing wave rate. When a large number of lobes is present, each lobe is shorter, as a rule, than those of males with a smaller number. This acts, of course, in a full-grown animal, to keep the total functioning volume of the testis a constant as new lobes are added.

In the preceding consideration of the effect of wave rate it has been assumed that the time elapsing before lobule regeneration occurs has not been affected. This, however, is probably subject to the influence of metabolic conditions in the same way as is wave rate. We may safely assume, I believe, that lobule regeneration could be somewhat hastened by very favorable conditions or delayed by unfavorable ones. The effect in the first case would be to reduce the length of the interval between lobes, and therefore permit the establishment of a greater number; the effect in the second case would be of the opposite nature.

Favorable metabolic conditions, therefore, would tend to increase the wave rate and through it the size of the lobes, reducing at the same time their possible number; the same conditions, however, would probably cause earlier regeneration, shorten the interval between lobes, and tend thus to permit a greater number. Unfavorable conditions, by slowing the wave rate, shorten the lobes and thus increase the number possible; the same conditions, though, would tend to delay lobule regeneration and the formation of new lobes, increase the interval between lobes, and reduce the number. The net change, so far as the functional volume of the testis is concerned, is small; and excessive increase or reduction of the total output of reproductive cells will not occur, in a fully developed male, except under very unusual conditions.

The writer, therefore, though agreeing that bodily vigor may influence lobe number, insists that it does so only by affecting the length of the primary germ-cell cord, modifying the rate of the spermatogenetic wave, or changing the time necessary for lobule regeneration. In other words, given a spermatogenetic pattern such that multiple testes are possible, the modification of this pattern, by whatever influences it may be brought about, inevitably modifies to some extent the end result. Hence one may expect a certain variation in lobe number even in animals of exactly the same age.

So, too, on this basis, the occasional differences between the testes of the individual male become understandable. Figures 22 and 23 illustrate the testes from animal number 437, killed on June 26. One testis (Fig. 22) shows a well-developed anterior lobe. In the other (Fig. 23) the disappearance of this lobe is practically complete. Nevertheless the almost microscopic enlargement containing degenerating lobules and interstitial cells shows that a functional lobe occupied this region in the preceding summer. At the caudal end of the testis represented in Fig. 23 a small lobe is developing; in the other (Fig. 22) only a slender caudal germ-cell cord appears. Clearly the one testis is a season in advance of the other, as evidenced both by the earlier disappearance of a lobe anteriorly and the earlier development of a new lobe caudally. Whether in the animal's first year of sexual maturity only one testis had become functional, or whether at some time later the spermatogenetic processes were retarded in one organ, it is impossible to state. Other animals have been found in which the two testes differ only in the size of the anterior lobes, they being of the types shown in Figs. 15 and 16. Still other males show differences only in the stage of development of the posterior lobes; animals are frequently found with one testis of the type shown in Fig. 4, while the other is as well developed as that in Fig. 5. Testes differing as do those of Figs. 5 and 6 have also been removed from a single individual. All the differences so far encountered, however, are but further evidence that the multiple testis is an expression of the spermatogenetic processes. Considering the lobes as structures of a segmental origin, or as divisions of the testis associated merely with the body size, the differences found in a single animal would be difficult of interpretation; realizing the manner in which lobes arise, run their course, and disappear, the occurrence of such differences is even to be expected. Though found in but a small number of the males studied, such differences are by no means rare; and, when found, they are to be looked upon as a normal result of the different modification in the two organs of those growth, degeneration, and regeneration processes which make a multiple testis possible.

In the writer's opinion, no particular phylogenetic significance attaches to this peculiar type of testis. It occurs in several members of the family Salamandridæ; Champy mentions it in numerous European tritons and salamanders, and I myself have observed it in our two American members of the family. If axolotl, on the other hand, possesses a multiple testis, as Champy states, it differs from its near relative, *Amblystoma punctatum*.[1] Among the Plethodontidæ, too, *Desmognathus* stands alone, no other member of the family, to my knowledge, possessing a multiple testis. Since the plan of spermatogenesis in *Necturus* or *Cryptobranchus*—in fact, in any of the urodeles I have examined—differs from that in *Desmognathus* or the Tritons in no fundamental way, a multiple testis might arise in any of these urodeles if the spermatogenetic processes became sufficiently reduced in rate. In this way, probably, have the species now possessing multiple testes arisen from ancestors with organs of the simple type. Such slowing of the reproductive processes in phylogeny might possibly be interpreted as due to deterioration in the vigor of the stock; the writer sees neither in it nor in the resulting structure of the testis any particular adaptative value.

Summary and Conclusions.

1. Multiple testes of two or more enlargements or lobes separated by intervening non-functional regions are of common occurrence in *Desmognathus* and other urodeles.

2. Such multiple testes are observed only in larger males. The smallest sexually mature males have testes of but a single lobe.

3. A study of the manner in which new lobes appear indicates the following mode of origin:

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[1] Spengel says: "Beim Axolotl erscheint er (the testis) als eine breite, dicke, von zahlreichen Unebenheiten besetzte Platte." This description is somewhat more applicable to the testis of *Amblystoma punctatum*. Irregularities, however, are to be observed in the testis of *Amblystoma* only in the spring when the lobules are emptying, and are in no case so extreme as to be at all similar to the lobes of a true multiple testis. It may be questioned whether the "lobes" described by Champy in axolotl are similar in origin to those in *Desmognathus* and the tritons, or whether they are merely such "Unebenheiten" as Spengel mentions. No other investigator has described the testis in axolotl as being of the multiple type.

(*a*) The portion of the testis emptied in autumn is not regenerated immediately, since the residual spermatogonia remaining in the region do not begin division, but remain quiescent for several months.

(*b*) The functional testis in the next season develops anterior to the emptied region, from a part of the original germ-cell cord not developing spermatozoa in the first season.

(*c*) When the emptied lobules degenerate and disappear, the region they occupied remains as a slender strand or cord of residual spermatogonia.

(*d*) Spermatogonia in the caudal end of the cord finally begin division. Lobules of germ cells develop and a caudal "secondary testis" or lobe is formed.

(*e*) The second lobe shifts forward each season in the same manner as does the first. From the germ-cell cord left in its wake a third lobe eventually arises. The process is repeated in the case of other lobes subsequently added.

(*f*) The most anterior lobe finally reaches the cephalic end of the germ-cell cord and disappears. Other lobes follow in due time, but are replaced by new ones originating caudally.

4. New lobes have been found to appear in no other manner than by the process above outlined.

(*a*) Sexually immature males never show numerous growth centers in the germ-cell cord.

(*b*) New lobes do not arise by the promiscuous establishment of secondary growth centers between lobes already present.

(*c*) Small anterior lobes always prove to be disappearing or "running out"; new lobes have not been found to develop in this region.

5. The conditions necessary for the formation of a multiple testis are:

(*a*) A slow forward movement of the spermatogenetic wave.

(*b*) Delayed regeneration of the emptied lobules.

(*c*) The lapse of a time interval following which the long-postponed regeneration brings into existence the new lobe. The unregenerated region between lobes is that commonly termed "sterile."

6. The multiple testis is clearly but a result of the spermatogenetic pattern of the species possessing it.

7. Modifications of the spermatogenetic pattern cause differences in the number or size of the testicular lobes even in animals of the same age. Even in the individual the two organs, from a similar cause, may occasionally be of different type.

8. Neither the multiple testis nor the peculiar combination of factors producing it are regarded as of important phylogenetic or adaptative significance.[1]

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THE HYDROLYSIS OF HIGHER FATS IN EGG–SECRETION.

OTTO GLASER.

I.

In four recent publications ('15, '18, '21, and '22) Richards, Miss Woodward, and I have dealt with the enzymic properties of *Arbacia* egg-secretion. It was shown, first of all, that radiation affects these secretions as though enzymes were present; next, there was precipitated a body, lipolysin, which, like unmodified exudate, was subsequently shown to accelerate the hydrolysis of ethyl butyrate. Finally, in my last paper ('22), I reported the synthesis of butyric ester.

On the basis of these results, we may assume that lipolysis plays a rôle in the initiation of development. The position is greatly strengthened by the fact that normal eggs may be completely sterilized if their secretions are removed by short exposure to charcoal ('21). Nevertheless the view that initiatory changes are somehow linked with the activities of a lipolytic enzyme requires further evidence. So far the results with ethyl butyrate are the clearest of all. However, if these were due, as they might be, to a relatively specific esterase, the fact, though interesting from other points of view, would be difficult to fit into a theory of fertilization. In order that lipolysis may find a place as part of the mechanism of initiation, it must first be shown that the enzymes present in egg-secretion actually affect the hydrolysis of higher fats.

II.

Preliminary tests were made with olive oil. This was carefully neutralized with dilute NaOH and subsequently extracted with fresh water, and later with ether ('09). The oil was then ready for use.

If egg-secretion influences the hydrolysis of olive oil, the effect, under the proper conditions, might be rendered apparent through changes localized at the oil-exudate interface. Here important disturbances should take place, although mere volumetric changes are not likely to be reliable. The diffusion of the glycerine set free in hydrolysis would, of course, result in a decrease in the volume of the oil drops. On the other hand, the production of oleic acid with its great affinity for oxygen and its capacity for breaking down into lower fatty acids might easily balance, or even overbalance, the loss resulting from the solubility of the glycerine in egg-water. Conceivably, then, the drops might decrease in volume, increase in volume, or even remain constant.

Nevertheless, if hydrolysis takes place, a lowering of the surface tension at the oil-exudate interface is to be expected, and, regardless of volumetric considerations, should bring about a concentration at the phase-boundary of substances either in solution or in suspension within the oil. If such substances happen to be insoluble in egg-secretion, one may likewise expect their precipitation immediately about the periphery of a drop from which glycerine and possibly the oxidation products of oleic acid, are diffusing outward.

I used two indicators; in some tests Sudan III. was dissolved in the olive oil, in others pulverized charcoal was suspended. A sharp phase-boundary, permitting a clear focus under the microscope, was secured by placing small discs of the oil on the surface of the exudate. The observations were checked by comparisons made on sea-water and distilled, as well as by the use of oil without indicators. Additional controls were carried on with solutions of pancreatin and the commercial Holadin.

With discs of plain olive oil on sea-water, there is some marginal effect. This is indicated by the rather rapid development of a moderately irregular outline. On distilled water the discs maintain their smooth circular form almost perfectly during the first few hours of an experiment. The differences in these two cases are no doubt traceable to the sea salts.

If egg-secretion is used in place of sea-water, the oil discs in the course of an hour take on a form suggestive of a circular saw whose projecting teeth are in a state of active disintegration. Small particles can be seen breaking off in large numbers until the originally sharp outline of the disc becomes quite obscured. Similar effects were noticed on sea-water, but they develop less rapidly and, within the time limit of these experiments, never equaled the effects gotten with egg-secretion.

With Sudan III., in the early stages of an experiment, peripheral elimination and precipitation of the dye on either sea-water or distilled is questionable. On egg-secretion, however, there is no doubt. The outlines of the discs become exceedingly irregular; there is marked surface concentration of the stain, and very minute particles of it are precipitated densely about the periphery. After 12 hours the oil discs are quite gone. In place of them one finds irregular islands, stained with Sudan III. After 24 hours a rancid odor is noticeable.

With pulverized charcoal exactly comparable results can be gotten, only the surface changes are even more striking. After 24 hours the islands on egg-water can be distinguished macroscopically from their controls, partly because of their greater irregularity, and partly because they have become vesiculated.

If the differences noted are due to the activities of a lipolytic ferment, then solutions known to contain fat-splitting enzymes should reproduce these differences. Attempts were made with two products, the one labeled Pancreatin and of unknown origin, the other a Parke-Davis preparation with the trade name Holadin. Both of these, especially in experiments in which charcoal was used, gave very striking effects and, after 24 hours, the irregular vesiculated islands could be distinguished even macroscopically from their controls.

III.

In these first experiments I made no attempt to control bacterial action. Whatever may be true after six, twelve, or twenty-four hours, the earliest noticeable differences between the oil discs on exudate and on sea-water became apparent so quickly that bacterial digestion seems unlikely. This fact, therefore, warrants a more careful examination of the fat-splitting properties of the secretion.

I now prepared exudate as free as possible from bacterial contamination. The females were thoroughly washed in running fresh water and carefully dried. Only shed eggs were used and these in filtered sea-water. The secretion itself was filtered several times through Chardin paper and the remaining infection controlled by the addition of chloroform. KCN was not employed because it checks the action of certain lipases.

With exudates of this type I shook up varying proportions of either olive oil or whale oil and at stated intervals attempted to titrate the systems with NaOH N/40 back to the specific turning points of such indicators as phenolphthalein, neutral red, or litmus. On account of the buffer effects and saponifications due to the salts present in both secretion and sea-water, such titrations, even if otherwise reliable, can not disclose the total acidity.

But there are other difficulties. Digests of the type here under consideration are almost proverbially mean. In addition, these particular systems in egg-secretion, in the course of an hour or two, underwent serious physical changes. At the beginning of an experiment, oil and secretion, after shaking, would separate promptly, but after standing for the stated times at 20° C. the separation was much less complete. After four hours even moderate shaking imparted to the systems a stability almost jelly-like. Under these circumstances, even with the addition of neutral alcohol ('03), titration gave highly variable results, and hence nothing in the nature of a curve showing the course of the reaction can be plotted. However, if we take the most reliable titrations—*i.e.*, those made upon volumes measured before the physical changes previously referred to had taken place—certain very definite comparisons are possible.

Series *A*.........	40 c.c. secretion 10 c.c. olive oil	40 c.c. sea-water 10 c.c. olive oil
After 45 hrs......	10 c.c.=2.7 c.c. NaOH $N/40$	10 c.c.=1.9 c.c. NaOH $N/40$
Series *B*........	150 c.c. secretion 25 c.c. olive oil	150 c.c. sea-water 25 c.c. olive oil
After 30 min......	10 c.c.=1.5 c.c. NaOH $N/40$	10 c.c.= .4 c.c. NaOH $N/40$
Series *C*.........	90 c.c. secretion 25 c.c. olive oil	90 c.c. sea-water 25 c.c. olive oil
After 30 min......	10 c.c.=1.8 c.c. NaOH $N/40$	10 c.c.=1.3 c.c. NaOH $N/40$

These values indicate the number of c.c. of NaOH N/40 necessary to return the several systems to P_{H_8}, using phenolphthalein as the indicator. The relatively high acidity found in the controls is a disturbing factor, but unavoidable, since oleic acid is never entirely absent in a "neutral" oil; is, moreover, constantly being produced; and, as constantly oxidized to dioxystearic and, very likely, other lower fatty acids. The differences between the digests and the controls, however, remain significant.

IV.

By far the most fruitful observation was made, not on the digests themselves, but on the litmus which in certain cases was used as the indicator. As compared with the controls in sea-water and oil, and in exudate without oil, neutral litmus, when shaken up in mixtures of secretion and either whale oil or olive, instantly becomes pinker. After two hours the pink is distinctly intensified, and after twelve, such digests stand out sharply from their controls.

Within twenty-four hours all color, including the pink, disappears in the oil-secretion digests. If more litmus is now added, it again turns pink, and, in the course of time, fades out completely. The controls, on the other hand, even after forty-eight hours, still appear "neutral," with perhaps only the faintest leaning toward pink.

It is plain that the litmus is not functioning here as a direct indicator of acidity. Very likely the change to pink and the final complete decolorization are the first and last visible steps in a process of reduction. But why, we may ask, the difference between the secretion digests with neutral oil and the controls? If the explanation is to be found in the superior reducing powers of the oil-secretion systems, then these must be producing a substance capable of binding oxygen. Moreover, they must be producing this material at a rate far in excess of the rate in the controls.

Now, if the oils in the presence of secretion are undergoing ordinary lipolytic cleavage, one of the reaction products must be oleic acid, and this, as is well known, absorbs oxygen with great ease. Is it possible, then, to attribute the decolorization of the litmus to the reducing powers of oleic acid? We can only do this if we can first show that oleic acid is present in higher concentration in the digests than in the controls; and, if we succeed in proving this, we shall incidentally also furnish the proof that the only source of oleic acid—the neutral oil—is undergoing accelerated hydrolysis in the presence of egg-secretion.

V.

With this idea in mind, I prepared a special set of tubes, using two volumes of egg-secretion, one volume of either neutral olive or whale oil, and one volume of a neutral litmus solution. The tubes were "chloroformed" as before, then stoppered and shaken.

The secretion used in these tests was made with every precaution possible, and I kept a microscopic check on each tube. In a few instances I could find no organisms whatever either at the beginning or at the end of the period of digestion; in other cases the number was too slight to account for the results, since control tubes, deliberately infected to the point of cloudiness, required forty-eight hours to produce a barely noticeable reduction of the litmus, whereas in the oil-secretion digests the reduction always began instantaneously.

Tubes without chloroform became turbid within forty-eight hours and developed the unmistakable rancid odor. In the tubes with bacterial growth inhibited no rancid odor could be noticed on account of the masking effect of the chloroform. This necessitated some other indicator for the presence of oleic acid.

The test finally chosen was based on a statement of Hammarsten's ('12), and depends on the fact that oleic acid in the presence of cane sugar and sulfuric acid develops a purple color. The usual method was to place a drop of the digest on a slide, to mix with this a drop of saturated solution of cane sugar, and to add from 2 to 5 drops of concentrated sulfuric acid.

Care must be taken not to add the sulfuric too quickly, or to use too much, for if the sugar breaks down suddenly, the carbon set free obscures the reaction in the oleic acid. With these precautions, however, the test is delicate and very reliable. The change in the oleic-acid globules can easily be followed under the microscope. It begins as a slight discoloration, succeeded by pink—rose—and finally a deep purple. At 20° C. the reaction is slow with freshly formed oleic acid, but proceeds more rapidly after the oleic has been exposed for some time to the air.

By this method it was possible to demonstrate the presence of oleic acid in the digests in which chloroform had masked the rancid odor. Moreover, the concentration of oleic acid, as shown by the number, size, distribution, and depth of color of the purple globules, left no doubt that hydrolysis was proceeding at a faster rate in secretion than in sea-water. In the controls an occasional globule did turn purple, but this was to be expected from our inability to prepare, or, for that matter, even to keep, a fatty oil absolutely neutral.

VI.

It may be taken as demonstrated, then, that *Arbacia* egg-secretion has the power to hydrolyze higher fats. Since neither whale oil, olive oil, nor ethyl butyrate occur in sea-urchin eggs, we must conclude that the lipase present is non-specific.

The question now remains whether the lipolysin first isolated by Miss Woodward also affects the hydrolysis of higher fats.

To determine this, I repeated the experiments described in the preceding section, using, in place of egg-secretion, sea-water solutions of the lipolysin precipitate. The concentrations employed were, roughly, 25 milligrams in 10 c.c. of solvent. To report the results in detail would be a mere reiteration of the experiments with egg-secretion. In every case the evidence for hydrolysis was positive, and leads to the conclusion that the precipitated lipolysin is, or contains, that enzyme which in unmodified egg-water is responsible for the hydrolysis of the higher fats.

Amherst College,
February 4, 1922.

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BIOLOGICAL BULLETIN

THE EFFECT UPON DEVELOPING EGGS OF EXTRACTS OF EMBRYOS OF THE SAME SPECIES.[1]

MARY GRACE SPRINGER.

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INTRODUCTION.

The investigations described in this report are an attempt to isolate formative substances from the early embryological stages in the developing egg of *Arbacia punctulata.* Upon the hypothesis of formative stuffs, egg extracts should show the presence of such materials, if under the conditions of the experiment they are isolable. Since development is conceived of as a series of exceedingly complex reactions involving both chemical and physical factors, the formative substances present in the fertilized egg must be the substances which begin this long sequence of reactions. If by their action and interaction, other and possibly more complex substances are synthesized, then at a given stage in development we should expect to find in the extracts of the larvæ the formative substances of that period, provided of course they go into solution in the solvents used. If such is the case, it seems decidedly important to find out whether by treatment of fertilized eggs with extracts of larvæ at a given stage, it is possible to accelerate the development of the eggs when they reach that specified stage. The cell walls of the developing eggs presumably must be permeable to the formative stuffs present in the solution, if any effect is to be registered.

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[1] Contribution from the Zoölogy Department, Oberlin College and the Marine Biological Laboratory.

The whole of the work was done at the Marine Biological Laboratory at Wood's Hole during the summer of 1921. The writer is indebted to Professor C. G. Rogers for suggesting the problem and for his criticism of the plan of work.

Methods and Materials.

The animals used in these experiments were the starfish, *Asterias forbesii,* and the sea-urchin, *Arbacia punctulata.* The eggs and sperm of both were secured by shaking the ovaries or testes into a dish of sea water. The starfish eggs were filtered through silk bolting cloth to remove portions of the ovarian tissue. The animals were first washed under a stream of fresh water and all precautions were taken to cleanse thoroughly the glassware and instruments used.

By "egg extract" is meant a suspension of crushed eggs in sea water or other specified solvent. The eggs were centrifuged, the fluid decanted, and the eggs ground up with fine sea sand, free from impurities, and thoroughly washed by the solvent used.

In some experiments the eggs were crushed between glass plates, but this method was not satisfactory. No uncrushed eggs were present in the solutions to invalidate the percentages. Extracts of larvæ were made up in the same manner. To maintain as uniform concentration as possible, a definite proportion of eggs to solvent was kept (1 c.c. eggs to 5 c.c. solvent) but the concentration was probably lowered by the retention of some of the protoplasmic fragments in the sand.

The term "blastula water" or "gastrula water," for example, is understood to mean water in which these larvæ have stood up to that stage in their development. Both the eggs and sperm in any one experiment were derived from one individual each whenever possible. The sperm suspension used was concentrated enough to cause 100 per cent. fertilization.

Data of the exact time of the 2-, 4- and 8-cell cleavages chiefly are noted. Although it is difficult to say just when, in terms of actual minutes after fertilization, the late gastrula becomes a pluteus for instance, by careful comparison of the culture with the control one can observe any differences in their stages of development.

Two methods of comparing rates of cleavage were employed: the time elapsing between insemination and the first 2-, 4- or 8-cell stage, or the period between insemination and the time the culture showed a 50 per cent. development of a given stage. For obvious reasons the former method was used for the most part, only the time required for making a single observation elapsing between the readings, and, in order that there might be no errors introduced merely by the arrangement of the culture dishes, the order of readings was frequently reversed. Equal quantities of eggs from the same suspension were used for each culture to insure an equal opportunity for oxidation and presumably then an equal concentration of CO_2 in the water. The cultures were kept in flat-bottomed finger bowls and were stirred frequently. During the season the temperature of the room varied from 21° to 23.5° C.; the variation in the temperature of the sea water in the circulation as recorded each day was found to be from 18.1° C. to 20.5° C. The pH of the sea water was tested each day and was found to be very constant—8.2 or at a few times 8.0 when thymol blue was the indicator used. Whenever the quantity of extract was sufficient to admit of its being tested, the pH was found to be 8.0 or slightly more acid. It did not appear that there was a characteristic difference between the degrees of acidity noted in the extracts made of larvæ in different stages of development.

A new supply of animals was brought into the laboratory each day. These were kept in an aquarium placed on a cement water table with a stream of fresh water running into the aquarium constantly.

The basis of comparison between a normal and a retarded culture in all experiments was (1) rate of development, (2) size, (3) vigor (rate of movement), (4) longevity.

Sample tables are not given for the experiments on the starfish because the rates obtained for both the earlier and late stages were so varied that no definite conclusions can be fairly drawn from them. These variations were doubtless to be explained in part at least by the fact that toward the end of the breeding season the physiological condition of the eggs was far from uniform. In general it was found that the starfish cultures containing extract showed a slight retardation, a higher degree of cytolysis, and a greater tendency to stop either at the blastula or the gastrula stage.

All the extracts used in these investigations upon *Asterias* eggs were made in a solution of sea water.

The Effects of Embryological Extracts on the Early Stages of the Sea-Urchin Egg.

The following experiments were all performed on the sea-urchin, *Arbacia punctulata,* to find out if the extracts of various larval stages have any effect on developing eggs of the same species. The eggs of *Arbacia* while more stable, and therefore less desirable than those of *Asterias,* can be obtained in convenient quantities for use in making extracts.

About sixty series of experiments were performed. The method was strictly comparative. It will be noted that the tables show, not only the effect of a given extract, but its effect in varying concentrations as well.

Particular emphasis is laid on the fact that the experiments given in outline in the table are type experiments. Each experiment outlined is one of several very similar experiments unless otherwise stated and is taken as fairly representative of the results obtained in all the experiments of that series, the others of which are omitted to avoid unnecessary and tedious repetition.

Series 15.

Table I.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.	pH of Extract.	Cleavage Rate.		
							2-cell Stage.	4-cell Stage.	8-cell Stage.
A....	50 c.c.	25 c.c.	Gastrula	18.2° C.	8.2	8.0	44	92	117
A′....	50 c.c.	25 c.c.	"	"	"	"	49	97	114
B.....	75 c.c.			"	"		48	75	98
B′....	75 c.c.			"	"		47	71	96

Note.—In this particular experiment the figures given under cleavage rate are in terms of minutes which elapsed between insemination and the appearance of the first 2-, 4- or 8-cell stage. It should be noted that the amount of extract used in this series was relatively large. (pH significance discussed later in paper.)

Later Development and Fate.—The eggs in cultures *A* and *A′* stopped at the 128–256-cell stage, and a very general cytolysis occurred.

The eggs in *B* and *B′* developed into vigorous normal plutei.

Conclusion.—The table shows that there was a definite retardation at the second and third cleavages in the cultures containing extract, and a complete arrest of development at the beginning of the early non-motile blastula stage.

Series 17.

TABLE II.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.	pH of Extract.	Cleavage Rate.		
							2-cell Stage.	4-cell Stage.	8-cell Stage.
A....	25 c.c.	25 c.c.	Blastula Water	18.4° C.	8.2	8.0	50	84	89
A′....	50 c.c.			"	"		50	67	84
B.....	40 c.c.	10 c.c.	Motile Blastula	"	"	"	51	68	89
B′....	50 c.c.			"	"		49	66	83
C.....	45 c.c.	5 c.c.	"	"	"	"	51	77	81
C′....	50 c.c.			"	"		51	67	83
D....		50 c.c.	Blastula Water	"			68	106	138
D′....				"	"		48	65	84

Note.—In this experiment the figures given under cleavage rate are in terms of minutes which elapsed between insemination and the presence in the culture of fifty per cent. of the stage indicated.

Later Development and Fate.—Development in cultures *B, C,* and to a slight degree in *A,* was definitely retarded, especially between the gastrula and early pluteus stages. In the great majority of cases the gastrulæ did not become plutei at all. The eggs in culture *D* were very much retarded; in fact, few progressed beyond the blastula stage, due probably to lack of oxygen in the water, and possibly to a high concentration of carbon dioxide. Many of the eggs in the controls developed into plutei.

Conclusion.—The table shows a very slight retardation in the early cleavage rate of *A, B* and *C,* and a greater amount of retardation in the later stages, especially at the early gastrula stage. *D* was very markedly retarded.

A very unusual proportion of exogastrulæ was found in all the cultures. There was even a small proportion in the controls. The reason for this is unknown. It happened that this particular series of experiments was carried on during the hottest days of the summer, but the bowls were kept in the water table, and the temperature of the room was not above 23° C. Later experiments failed to show an equally high percentage of exogastrulæ, although they were carried on under conditions as nearly identical as possible. The only explanation to be offered is that there may have been some variation in the eggs of the particular female used which caused them to evaginate instead of invaginate.

The question of whether these abnormal cultures of exogastrulæ could possibly be influenced to develop normally if extract of larvæ at the same stage was added came up at this point. Of course it is obviously improbable that in making the extract one would at the first trial secure larvæ at the identical point of development of those in the culture. The eggs in the culture were, however, centrifuged, the stale water decanted, and fresh water added. Numerous trials of extracts made up of embryos at different points in the same general developmental stage, and in preceding and succeeding stages were used. In no case did the exogastrulæ proceed with their development.

Series 32.

TABLE III.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.	pH of Extract.	Cleavage Rate.		
							2-cell Stage.	4-cell Stage.	8-cell Stage.
A....	50 c.c.	15 c.c.	Pluteus	18.6° C.	8.2	8.0	62	118	131
A′....	65 c.c.			"	"		55	79	98
B.....	50 c.c.	10 c.c.	"	"	"	"	62	96	116
B′....	60 c.c.			"	"		54	77	99
C.....	50 c.c.	5 c.c.	"	"	"	"	57	79	120
C′....	55 c.c.			"	"		56	78	96
D....	30 c.c.	20 c.c.	Pluteus Water	"	"		57	84	104
D′....	50 c.c.			"	"		56	80	98

Note.—The cleavage rate is given in terms of minutes between insemination and a fifty per cent. development of the specified stage.

Later Development and Fate.—The controls developed faster and more normally than did the other cultures, which were all retarded in the early cleavage stages and at the non-motile blastula stage. All the cultures finally developed into gastrulæ. Some exogastrulæ were found in all the cultures containing extract. No development beyond the late gastrula or very early pluteus stage was observed in the cultures containing extract.

The controls showed normal development.

Conclusion.—Definite retardation was noted in the cultures containing extract and total arrest at the late gastrula or early pluteus stages.

Series 26.

TABLE IV.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.	pH of Extract.	Cleavage Rate.		
							2-cell Stage.	4-cell Stage.	8-cell Stage.
A.....	40 c.c.	12 c.c.	Water fr. 128–256 cell stage	18.6° C.	8.2	8.0	44	77	91
A	52 c.c.			"	"		36	70	86
B.....	50 c.c.	5 drops	128–256 cell stage	"	"	"	44	72	85
B′....	51 c.c.			"	"		39	70	84
C.....	50 c.c.	10 c.c.	"	"	"	"	49	76	93
C′....	60 c.c.			"	"		37	68	81

Note.—Under "cleavage rate" is noted the number of minutes which elapsed between insemination and the appearance of the first 2-, 4- or 8-cell stage.

Later Development and Fate.—Development to the gastrula stage was nearly parallel in all the cultures. *A* did not develop into very vigorous plutei; *B* showed plutei a trifle smaller than normal, while in *C* were found plutei, but many abnormal types. All the controls had normal plutei.

Conclusion.—Slight retardation in cultures containing the extract or the "water."

Several experiments were performed using instead of extracts, water in which eggs had developed. As above specified, "blastula water" is understood to mean water in which eggs have developed to the blastula stage. Details of these experiments are not given because the results do not differ in any way from those obtained by the use of such water as indicated in the above tables. If a relatively large amount of blastula water, *i.e.*, 15–20 c.c. to 50 c.c. of sea water is used, a very slight retardation is noticed when the culture is compared with the control. If only very small amounts of this "water" are employed no perceptible retardation occurs. Since these results seemed to indicate that possibly the retardation in the case of the eggs developing in the blastula water, for example, was due simply to an insufficient supply of O in the water together with increased acidity, boiled sea water was substituted for the blastula water. A very slight retardation was observed—not so evident by any means as that seen in the controls employing blastula water, and quite within the limits of experimental error. This decrease in the degree of retardation, however, may have been due in part, at least, to a decrease in the number of bacteria present in the cultures made up with the boiled sea water.

Since in all the experiments it was quite evident that there was a shorter length of life in the cultures in which extracts were used, an experiment was made using extracts of suspensions which had been boiled previous to being centrifuged, and comparisons drawn

Series 36.

TABLE V.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.	pH of Extract.	Cleavage Rate.		
							2-cell Stage.	4-cell Stage.	8-cell Stage.
A....	100 c.c.	30 c.c.	Blastula (boiled)	19.7° C.	8.2		55	100	—
A′....	130 c.c.			"	"		45	99	—
B.....	100 c.c.	10 c.c.	"	"	"		50	95	—
C.....	100 c.c.	5 c.c.	"	"	"		49	81	—
D.....	100 c.c.	30 c.c.	Blastula Water	"	"		52	82	—
E.....	100 c.c.	10 c.c.	"	"	"		51	80	—
F.....	100 c.c.	5 c.c.	"	"	"		48	78	—
F′....	—	100 c.c.	"	"	"		—	—	—

with the results obtained by the use of unboiled extracts. Boiling the suspension to be used for extract was done not only to destroy bacteria, but also to take into account the bare possibility that substances in the larvæ which were evidently not soluble in cold sea water might go into solution in hot water.

Note.—Cleavage rate given in terms of minutes which elapsed between insemination and the appearance of the first 2-, or 4-cell stage.

Later Development and Fate.—Development in all the cultures was nearly parallel through the blastula stage except in *F′*, in which the eggs failed to segment at all. From the blastula to the pluteus stage the cultures containing extract were very slightly retarded as compared with the control *A′*. There was also a very slight retardation in the cultures containing the specified amounts of blastula water. In all cases, as usual, the retardation resulted in the production of plutei varying slightly from the normal in many respects, but yet producing no one definite type of abnormality.

Boiled extracts of gastrula and early plutei were made also, but the results obtained were entirely similar to those indicated above.

Conclusion.—Boiling the extract appears to reduce, if not to eliminate entirely the amount of retardation in the cultures.

Two other methods of making extract in a solution of sea water were tried, since it is obvious that if the extracts were made in a solution of the same osmotic pressure as that in which the eggs developed, that is, sea water, the problem would be much simplified.

The first variation in the method of making the extract was as follows: The eggs were centrifuged, and after the supernatant fluid had been decanted they were placed in a celloidin tube containing distilled water and set in a beaker of sea water. The eggs swelled and burst, presumably setting free substances which, if soluble in water, would dialyze through the celloidin tube into the sea water in the beaker. Extract made in this way was used, but the results obtained were not positive enough to be of value without further experiment. The "double sea water" method was also employed in one series of experiments with equally inconclusive results. No results were obtained, however, which were in any way contradictory to those previously noted.

Series 56.

An experiment was made, using motile blastulæ for extract, but instead of sea water, distilled water was employed, and the crushed eggs left over night (12 hours) in the distilled water before the extract was added to the cultures.

TABLE VI.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.
A......	50 c.c.	5 c.c.	Motile blastula	20.3° C.	8.2
A′.....	50 c.c. sea water 5 c.c. distilled water	—		"	"
B......	50 c.c.	3 c.c.	"	"	"
B′.....	50 c.c. sea water 3 c.c. distilled water	—			
C......	50 c.c.	1 c.c.	"	"	"
C′.....	50 c.c. sea water 1 c.c. distilled water	—		"	"

Later Development and Fate.—All the cultures and the controls appeared to reach the 128–256-cell stage at approximately the same time. A reading made four hours and fifteen minutes later showed *A* retarded, non-motile blastulæ; *A′*—blastulæ—a few motile. In *B* and *B′*, there were blastulæ, motile in both, but more vigorous in *B′*. No difference appeared at this stage in *C* and *C′*, in both of which the blastulæ were motile.

In the readings made on the following day, it appeared that *A* had stopped at the non-motile blastula stage. In *A′* were found vigorous gastrulæ. In *B* there were only a very few blastulæ which were moving while *B′* showed very vigorous late gastrulæ. No difference was noted between cultures *C* and *C′*. Both of these showed vigorous late gastrulæ.

Conclusion.—The cultures containing an appreciable amount of extract exhibited a retardation at the early non-motile blastula stage—an effect which was carried over into the later stages also.

Although the results tabulated above seemed to be practically conclusive, the question arose as to whether it might not be interesting as well as worth-while to use extracts made up in solvents other than sea water. Experiments made with such extracts should be of use as a check upon the other experiments. Chambers has contended that when a cell is broken into fragments by mechanical means, the small pieces, because of surface tension, usually become spherical in shape, and form a new surface film. Perhaps, then, by breaking down such a possible film by the use of solvents other than water, more of the "formative stuffs" might go into solution.

Series 47.

The extract in this experiment was made by placing the crushed embryos, motile blastulæ, in faintly acidified distilled water. The extract was allowed to stand for twelve hours before it was used. The acidified water was made by adding four drops of glacial acetic acid to 100 c.c. of distilled water.

The controls were, of course, made by adding equal quantities of the acidified water to the sea water when certain quantities of extract were used in the cultures.

TABLE VII.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.
A........	25 c.c.	5 c.c.	Gastrula	19.6° C.	8.2
A′.......	25 c.c. sea water 5 c.c. acid water	—		"	"
B.......	25 c.c.	3 c.c.	"	"	"
B′.......	25 c.c. sea water 3 c.c. acid water	—		"	"
C........	25 c.c.	1 c.c.	"	"	"
C′.......	25 c.c. sea water 1 c.c. acid water	—		"	"
D.......	50 c.c.	5 c.c.	"	"	"
D′......	50 c.c. sea water 5 c.c. acid water	—		"	"

Later Development and Fate.—Development in the cultures and controls was very nearly parallel until the blastula stage was reached. At this point, however, a very decided retardation in all the cultures containing extract was noted.

C was only very slightly retarded, however, at the blastula stage, and reached the pluteus stage very shortly after *C′*.

B and *A* showed many extreme modifications of form. Only a few plutei developed. *A′* and *B′* showed plutei better developed. In all the other cultures with and without extract, plutei were found, although many variations from the normal type were noted.

A separate control of eggs in sea water was kept. This showed a general retardation of development in all the cultures and controls containing even a small per cent. of acid.

Conclusion.—In the cultures containing extract an added retardation was shown when comparison with the controls was made. This retardation occurred chiefly at the early non-motile blastula stage.

Series 59.

In this experiment the extract was made of ciliated blastulæ in a solution of ether and water (1 part of ether to 2 parts of water, distilled). The water was used merely because it is very difficult to wash the extract from the sand by ether. Since water and ether are only very slightly miscible, the extract had to be shaken very vigorously before it was used.

Table VIII.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.
A......	50 c.c.	5 c.c.	Ciliated blastula	19.7° C.	8.2
A′.....	50 c.c. sea water 5 c.c. of ether-water mixture	—		“	“
B......	50 c.c.	2 c.c.	“	“	“
B′.....	50 c.c. sea water 2 c.c. of ether-water mixture	—		“	“
C......	50 c.c.	1 c.c.	“	“	“
C′.....	50 c.c. sea water 1 c.c. of ether-water mixture	—		“	“

Later Development and Fate.—*A* and *A′* showed very irregular cleavage from the first. Development in *B* and *B′*, *C* and *C′* was very nearly parallel through the motile blastula stage, but *A*, *B*, and *C* showed retardation in their development from the blastula stage to the pluteus stage. When the cultures were examined later, *C′* had many normal plutei; *C* had just a few small plutei, swimming feebly. *B* and *A* were retarded in their development, with fewer and smaller plutei than those in *B′* and *A′*. *A* showed by far the largest number of abnormal forms.

Conclusion.—A retardation was noted especially between the blastula and the pluteus stages in the etherial extract cultures.

Series 45.

The extract used in this experiment was made by soaking the embryos (motile blastulæ) after the usual crushing process, in a solution of one part of acetone in one part of distilled water, for 12 hours. This same solution was used in preparing the controls, and was always shaken vigorously before using.

Table IX.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.
A.....	50 c.c. sea water 5 c.c. 50 per cent. acetone	—		19.9° C.	8.2
A′.....	50 c.c.	5 c.c.	Motile blastula in 50 per cent. acetone	"	"
B......	50 c.c. sea water 2 c.c. 50 per cent. acetone	—		"	"
B′.....	50 c.c.	2 c.c.	"	"	"
C......	50 c.c. sea water 1 c.c. 50 per cent. acetone	—		" "	" "
C′.....	50 c.c.	1 c.c.	"	"	"

Later Development and Fate.—Development in the cultures and the controls appeared to be very nearly parallel until the early non-motile blastula stage. At that point in development blastulæ, some swimming vigorously, were found in *A* while in *A′* most of the blastulæ were non-motile, although a very few were moving feebly. In *B* and *B′*, *C* and *C′* there was also an evident retardation, although it was not so great as that registered in the *A* and *A′* cultures.

On the following day these readings were made:

A—very early plutei; *A′*—a very few late gastrulæ; *B*—early plutei; *B′*—very early plutei; *C* and *C′*—no difference apparent. A few late blastulæ, but mostly early plutei in both.

Conclusion.—A definite retardation was noted in the cultures containing extract at the early blastula stage and afterwards.

Series 53.

Extracts made up in alcohol (C_2H_5OH) must be used in small amounts, or else the alcohol must be a very weak solution, for if the concentration of alcohol in the cultures reaches three or four per cent. so many deaths occur in the blastula stage that comparison with the controls is difficult. Five series of experiments proved unsuccessful on this account.

In the experiment outlined below the extract was made up in 15 per cent. alcohol, and allowed to remain in the tightly corked tube for 24 hours before using.

TABLE XI.

Culture.	Amount of Sea Water.	Amount of Extract.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.
A.....	50 c.c.	10 c.c.	Blastula	21.1° C.	8.2
A′.....	50 c.c.	10 c.c. of 15 per cent. alcohol	—	"	"
B......	50 c.c.	3 c.c.	Blastula	"	"
B′.....	50 c.c.	3 c.c. of 15 per cent. alcohol	—	"	"
C......	50 c.c.	1.5 c.c.	Blastula	"	"
C′.....	50 c.c.	1.5 c.c. of 15 per cent. alcohol	—	"	"

Later Development and Fate.—Very irregular cleavage in *A* and *A′*. No apparent difference between *B* and *B′*, *C* and *C′* at the blastula stage.

Later: *C′*—many normal plutei.
C—a few normal plutei.
B and *B′*—*B* very slightly retarded, with fewer and smaller plutei.
A and *A′*—as *B* and *B′*, with greater retardation in *A* than in *B*.

Conclusion.—Since alcohol, even in very weak concentrations, has such a powerful effect on the developing eggs, no very definite conclusion can be drawn from this series of experiments.

Series 63.

In the table below are given some observations made in sea water whose OH concentration had been raised by the addition of 0.8 c.c. *N*/10 NaOH to 50 c.c. sea water. This experiment was also designed as a check upon the experiments in which normal sea water was used. Loeb ('98) brought out the fact that by allowing eggs, fertilized normally, to develop up to the blastula stage, and then dividing the eggs into three lots, to one of which NaOH is added in the proportion of 1.76 c.c. *N*/10 NaOH to 100 c.c. of sea water, a second to which the same amount of *N*/10 HCl is added, and the third for the control, at a given time the bowl containing NaOH shows complete plutei, the HCl culture shows late gastrulæ with a few short-armed plutei, while the control contains many plutei in various stages, and some late gastrulæ. In the report of this experiment Loeb attributed this result to an increased rate of oxidation produced by the OH-ion.[17] Now, on this hypothesis, if the retardation in the development of the eggs due to the presence of the egg extract is a slowing down of the oxidation rate, then presumably the NaOH should neutralize, or antagonize this effect, and we should expect a rate of development more nearly normal.

The experiments proved that just the reverse is true, however, for the effect of the NaOH was not to neutralize, but rather to add to the effect of the extract, since it can be seen by glancing at the table that, if anything, in the culture containing NaOH plus extract, there was a slight retardation in the early cleavage rates compared with the NaOH control. A more marked degree of retardation was shown in the development from the blastula to the pluteus.

This result is in entire agreement with the later work of Loeb.[18] In his book he says that although the rate of development of *Arbacia* can be retarded by the addition of acids to the sea water, he has not succeeded in showing that the rate of development in *Arbacia* eggs can be accelerated by the use of hydroxyl-ions in the sea water. Glaser found that this latter statement seemed to hold good for the early cleavages, since there was no definite increase in rate observed there, but that the rate of development from blastula to pluteus was accelerated. It would appear, however, that from Loeb's account of his experiments, the acceleration mentioned in the first paper was not observed until after the first day, so that there is after all no disagreement between the results obtained by these two investigators.

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[17] Glaser, Otto, "Qual. Analysis of Egg Secretions and Extracts of *Arbacia* and *Asterias*," BIOL. BULL., Vol. XXVI., No. 6, June, 1914.

[18] Loeb, Jacques, "Artificial Parthenogenesis and Fertilization," Chicago, 1913.

TABLE XII.

Culture.	Amount of Sea Water.	Amt. of Extract.	Amount of *N*/10 NaOH.	Kind of Extract.	Temp. of Sea Water.	pH of Sea Water.	Cleavage Rate.		
							2-cell Stage.	4-cell Stage.	8-cell Stage.
A . . .	50 c.c.	3 c.c.	1.75 c.c.	Blastula	21.9° C.	8.2	57	70	76
A′ . .	53 c.c.	—	1.75 c.c.		"	"	48	54	71
A″ . .	54.75 c.c.	—	—		"	"	40	58	70
B . . .	50 c.c.	1.5 c.c.	1.75 c.c.	"	"	"	53	64	72
B′ . .	51.5 c.c.	—	1.75 c.c.		"	"	50	55	69
B″ . .	53.25 c.c.	—	—		"	"	43	53	68
C . . .	50 c.c.	0.5 c.c.	1.75 c.c.	"	"	"	49	54	67
C′ . . .	50.5 c.c.	—	1.75 c.c.		"	"	51	57	64
C″ . .	52.25 c.c.	—	—		"	"	45	51	66

Note.—The time recorded under the cleavage rate is the number of minutes between insemination and the first appearance of the stages indicated.

Later Development and Fate.—It is evident that there is only a very slight retardation, if any, in the cultures containing the extract in small amounts during the early cleavages.

At a later reading *A* was found to contain non-motile blastulæ, *A′* motile blastulæ. In *B* and *B′*, *C* and *C′* no considerable difference was observed, except for the fact that in *B′* the blastulæ appeared to be swimming more vigorously than in *B*.

Still later, the reading showed the late gastrulæ in *A* dead while in *A′* were found some plutei of varying abnormality.

B—a few plutei, varying somewhat from the normal.

B′—flourishing plutei, varying somewhat from the normal.

C—as *B*; *C′* as *B′*.

The controls in sea water showed more rapid and normal development at all stages.

Conclusion.—A very slight retardation was produced by the use of the extract in the early cleavage stages. This effect was more evident from the blastula to the pluteus.

Experiments were also made using glycerine and chloroform for solvents, but since chloroform appeared to be very difficult to use, and since glycerine even in very small concentrations caused a high degree of mortality in the cultures, no further work was done at this point.

Analysis and Discussion of Results Obtained.

The end result of the investigations described in this report is that the larvæ of *Arbacia punctulata*, and probably of *Asterias forbesii*, contain substances which, when extracted in sea water or other solvents, *and when present in sufficient concentration,* retard the development of the eggs of the same species.

This retardation was shown in most cases only in a slight degree in the early cleavages, but more markedly in the later stages, especially at the early non-motile blastula stage. There would appear, then, to be certain periods at which the egg is more susceptible to the action of the extracts than at other times.

It is interesting to note in this connection that Lyon ('02) showed that eggs placed in a weak KCN solution gradually lose their resistance to the KCN as development proceeds. At the same time, there appeared to be certain periods at which the eggs were more susceptible to the poison than at other periods.

Child studied the effects of various chemical agents upon the egg of the sea-urchin to prove the existence of axial metabolic gradients as fundamental factors in the development of this form. While it was not at all proposed to attempt in these investigations a corroboration of Child's work, results in the controls in which the agents used as solvents for the larval extracts were the same as those which Child used, were similar, and many of the abnormal types which he described were noted.

Child[19] showed that the effect of KCN, C_2H_5OH, NH_4OH, NaOH, HCl, and CH_3COOH was a differential inhibition manifesting itself in various deviations from the normal course of

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[18a] Lyon, E. P., "Effects of Potassium Cyanide and of Lack of Oxygen Upon the Fertilized Eggs and the Embryos of the Sea-urchin (*Arbacia punctulata*)." *Am. Journ. Physiol.* Vol. VII., pp. 56–75, 1902.

[19] Child, "Larval Development in the Sea Urchin," *Journ. of Morph.*, Vol. XXVIII., 1916–17.

development, as, for example, the variations of the angle of divergence between the arms, the approach of the lateral parts to the median line, and in extreme cases fusion, and many differences in the proportions of the larvæ.

Child also endeavored to show that differential acclimation and recovery may take place, resulting in wide-angled plutei, and an increase in the size of the oral lobe with over-development of the anterior and median regions as compared with the posterior and lateral regions. No specificity in the different form changes produced by the different agents used was noted.

In the experiments described here, also, the deviations from the normal form of the embryos produced by the extracts were so varied that there seems to be no basis for claiming any specificity of action. All the evidence points not toward a qualitative, but a quantitative action of the extract as far as the normal processes of growth and development are involved.

Although it is impossible at this point to state whether or not the different extracts all produce the same effect on the developing *Arbacia* egg, the fact which is to be emphasized is that, however they act, or whatever processes of the egg are chiefly involved, the general effect of the extracts when present in sufficient concentrations is to retard or inhibit the fundamental metabolic processes in some way.

There is a definite normal rate of development for the eggs of each animal, yet this rate may be changed by various conditions of environment. The two most common modifying causes are a change in oxidation (which may be due to a variety of causes) and a change in temperature. Among others, Stockard[20] has shown recently in many experiments that a very wide range in the decrease of developmental rate is very easily brought about by even a slight change in the surrounding temperature or a reduction in the oxygen supply. He has also contended that a normal continuous development may be modified into a discontinuous one by stopping its course during a very early stage.

Now many variations from the normal were found to be produced by the use of the extracts in certain concentrations. Some of these were slight: others were more evident, as, for example, the exogastrulæ, noted in Table II. Their appearance may indicate an arrest of development at the gastrula stage or earlier; in fact, it was noticed that in most cases only after the larvæ had remained for a day at the gastrula stage, did the exogastrulæ appear. Some exogastrulæ, however, appeared after a slowing up of development from the blastula into the gastrula. The further records of experiments planned may render a more definite position on this point possible.

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[20] Stockard, C. R., "Developmental Rate and Structural Expression," *Amer. Journ. of Anat.*, Vol. XXVIII., No. 2, January 15, 1921.

As far as temperature was concerned in carrying out these experiments, it would seem that the comparatively small variation from day to day noted above in the discussion of methods, would scarcely be sufficient to cause the development of abnormal types. And furthermore, if by any chance slight changes in the temperature from day to day might have resulted in the development of abnormalities, these abnormalities should be present in the controls in as great numbers as in the extract cultures. Such was not the case in the investigations outlined in this report.

In considering the part which the extract may have played in altering the normal oxidations in the developing eggs, three possibilities are evident: the rate of oxidation may be increased—in which case it would seem more probable that there would be an acceleration rather than a retardation in the rate of development, or the rate of oxidation may be decreased by the action of substances in the extract upon the eggs, or yet again the rate of oxidation may be decreased simply because the extract itself contains substances which use up the oxygen in the water, and so deprive the eggs of their otherwise available supply. No facts can be brought forward at this time, however, to make any one, or two, or all of these possibilities appear to be the probable explanation of the phenomena under discussion.

It should be emphasized at this point that the retardation caused by the use of the extract results in arrests of development and the production of abnormal types very similar to those caused by the various chemical agents used, although it is not possible of course to state definitely that these result from disturbances of the same metabolic processes.

Another fact which should be emphasized is that there was very evidently a shorter length of life in cultures containing extract than in the controls. This lowered degree of vitality, or of resistance, often manifested itself in a slower rate of movement, particularly at the blastula stage. A possible explanation of this effect may be found in part in the increased danger of bacterial infection which the use of extract affords. The presence of the crushed, and disorganized protoplasm of the extract affords a splendid opportunity for the breeding of bacteria. Thus it must be remembered that in considering the causes of the retardation, and the malformations resulting from the use of the extract, the matter of bacterial infection and more especially the effects of the toxins produced by these bacteria should receive due emphasis. It should be stated, however, that the experiments in which boiled extract was used furnish some evidence, very slight though it may be, that something other than bacterial action is involved in the retardation and the malformation resulting from the use of the extracts.

SUMMARY.

1. Extracts of *Arbacia* larvæ in the 128–256-cell stage, in the early and in the late blastula, gastrula, and pluteus stages, when present in a sufficiently high concentration, definitely retard the development of eggs of the same species.

If these extracts are used in very low concentrations, the retardation may well lie within the limits of experimental and observational error.

2. The retardation noted is manifested slightly in the early cleavage rates, and more markedly in the later stages of development.

3. Besides retarding development, these embryological extracts often cause cytolysis, arrests of development, and a very noticeable failure of the eggs to develop beyond the early non-motile blastula stage.

4. The very evident tendency of the eggs to stop at the blastula stage suggests that possibly this stage is a stage peculiarly susceptible to the extract and characterized by a general lack of resistance.

5. It is possible that extracts made from larvæ in a certain stage differ qualitatively from those made of larvæ of an earlier or a later stage, but we do not know that this is true, nor do we know that the retardation is associated with any modification of the rate of enzyme action, or permeability, or oxidation, or any specific process. Probably it depends upon the great complexity of the protoplasmic system, and the fact that no one part of that system may be altered considerably without disturbing the equilibrium of that whole system.

6. It is not possible to say at this point that the retardation caused by the extract affects the same metabolic processes as do the KCN, CH_3COOH, NaOH, etc., but the use of the extract results in arrests and retardations of development such as are apt to be caused by these chemical agents, and the various types of malformations resulting are often similar.

7. This work does not indicate, then, either the presence or the absence of formative stuffs, but shows that under the conditions given they do not appear. Two possibilities present themselves. If formative stuffs exist, they would seem to be unable to pass into solution in sea water, in lipoid-soluble substances, and in solvents which are able to dissolve carbohydrates. Therefore, if present, they appear to be complex in character and in close association with the protein molecule. The other possibility is that these hypothetical substances may go into solution in certain solvents, but yet may not be able to register any effect upon the developing eggs, because the cell walls of the latter may not be permeable to the formative stuffs in solution.

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[3] Carnegie Institution of Washington Year Book 1918, p. 55, D. T. MacDougall. Report from the Department of Botanical Research, p. 55.

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[6] Wilson, E. B., "Experimental Studies on Germinal Localization," *Journ. Exp. Zoölogy,* Vol. I., p. 197–269.

[7] Conklin, E. G., "Organ-forming Substances in the Eggs of Ascidians," Biol. Bull., Vol. VIII., No. 4.

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[16] Conklin, E. G., "The Effects of Centrifugal Force on the Eggs of *Crepidula*," *Journ. Exp. Zoölogy*, Vol. XXII., No. 2, February, 1917.

DIGESTIVE ACTIVITY OF MESENCHYME AND ITS DERIVATIVES.

II. Proteins as Object (A. Edestin.)

VERA DANCHAKOFF and S. M. SEIDLIN,

Department of Anatomy, Columbia University.

1. Statement of the Problem.

The recently obtained results regarding the digestive activity of the adult splenic cells of the fowl upon two mammalian tumors, the Ehrlich sarcoma and the Crocker Fund tumor 180, have raised a series of problems. Owing to the complexity of the conditions involved in the experiments referred to, they could not be easily attacked and solved at that time.

Has the embryonic mesenchyme, found to be powerless against the proliferation of the two heterogenous tumors in the fowl, no power at all to digest foreign proteins in dead or living form? Is only the adult mesenchyme of the spleen to be regarded as a tissue endowed with a specific digestive function, or can any mesenchymal cell and possibly some of its derivatives, wherever found in the organism, exhibit under definite conditions one of the most fundamental powers of living matter, *i.e.,* that of digesting particulate proteins? Finally, the act of digestion performed by a group of cells, in a well-defined region of the organism, is it to be regarded as a purely local phenomenon, or as a process, the effects of which extend beyond the boundaries of the tissue directly involved?

These questions can be answered only in a fragmentary and insufficient way on the basis of already known data. Embryonic mesenchymal cells were occasionally observed to exercise a phagocytic and digestive action upon dead or weakened cells, but little is known as to whether they possess a similar power against foreign protein. Other than splenic mesenchymal cells have occasionally been observed in the adult to succeed in completely digesting heterogenous proteins, as for example, catgut of mammalian origin which is digested by the stroma cells of different animals. Though suggestive of a widespread digestive power of the mesenchyme, these observations do not allow any definite conclusions regarding its extent and its various aspects. Finally, the question has hardly been raised, as to whether a digestive activity temporarily developed by the mesenchyme or by its derivatives in a definite region, might not have a general effect upon the whole organism of the animal under experiment. In this connection, the production of induced immunity against tumors by introduction into the organism of particles of tissue of the same species as that bearing the tumor and the immunization against pathogenic bacteria by introducing the same organisms in a weakened state, is of interest. As to how a local process displayed in a well-defined region of the organism can influence its general properties and through what mechanism this can be effected, only few hypotheses and even less data can be found.

In order to approach the problems stated above, different heterogenous, non-injurious proteins should be introduced among stroma cells other than those of the spleen. The study of their digestion, especially when excessive amounts are introduced, would throw light upon the question as to whether easily mobilizable cells from remote parts of the organism will participate in this process along with the cells found in close proximity to the injected masses. The study of the digestion of the substances introduced should be pursued not only until its completion, but special care should be given to the fate of the phagocytes, and a method should be devised for identifying them in any part of the organism which they might enter, either in a passive way or by their own active movements. Rather small animals only can be used, the size of which would not offer insurmountable difficulties for the study of the final distribution of these phagocytes.

A favorable object for such studies has been found in the tadpole. Various amounts of different heterogenous proteins, insoluble in saline, such as edestin, fibrin, coagulated egg albumin, were introduced into the transparent tail. When it was found that enormous amounts of introduced material were easily digested within the tadpole tail, the details of this process and especially the structure and origin of those cells which exercised this activity were determined. When it was found that the region into which the material was introduced and which was promptly invaded after the experiment by numberless cells was as promptly abandoned by most of them, an attempt was made to determine their new whereabouts and to identify them. This part of the work has so far been only partly successful. The ultimate distribution of the phagocytes will be studied further in greater detail and the results given in another paper.

Material and Method Used.

The transparent tail of the tadpole has been frequently used not only as a convenient object for observing normal processes of growth and differentiation, but also as a medium in which the activity of the various stroma cells, under experimental conditions, could be studied with remarkable ease. Work on the tadpole tail has been done in this country chiefly by Eliot R. Clark and Eleanor Linton Clark. They studied in it the growth of vessels in vivo. They introduced under its epidermis microscopic particles of paraffin, India ink, croton oil and starch granules and studied the reaction called forth in the adjacent tissues by the presence of these foreign materials. Some of the materials used by them (paraffin and India ink) were of such a nature as would lead us to expect only a physical reaction from the adjacent cells. Among the other material used by them, the croton oil produced upon the adjacent tissue injurious effects such as it would upon any other living tissue. Uncooked starch granules produced no other effect than foreign bodies, while starch granules cooked to the point of gelatinization proved to be a powerful chemotactic agent for leucocytes. Ingestion of such starch granules took place, but digestion of it was not followed.

The result of this work did not permit of any decisive conclusions regarding the digestive activity of the tadpole stroma for the simple reason that most of the substances used are not digestible. If the same material were introduced into our regular digestive tract, no other results would be obtained, than those described by the authors in relation to the stroma cells of the tadpole. Paraffin would produce no direct effect upon the lining of the digestive tube, some of the granules of India ink might be incorporated by cells, if a sufficiently long contact could have been secured. Croton oil would produce an intensive inlammation, aseptic if applied by itself in a milieu free of bacteria. Starch granules would be digested, but even these only in certain parts of the digestive tract.

Our experiments consist of introducing various amounts of suspensions of different protein substances into the thin edges of the tadpole tail. The injections were made with extremely fine glass pipettes. After numerous more or less unsuccessful attempts to use rather complex and apparently well-devised methods of injecting, recourse was taken to the simple and efficacious way used by Doctor Clark and courteously demonstrated by him. The substances, suspended in saline solution, were blown into the tissue by lung pressure. The injections were made both into the dorsal and ventral edges of the tail. The epidermis of the tail is easily pierced in the caudal part of its edge by the fine end of a glass pipette containing a thick suspension of a fine pulverized protein, between its dorsal border and the axial strand of the denser tissue. The pipette is then pushed at about equal distance from dorsal and ventral borders of the transparent plate for about 2–4 mm. in the cranial direction. The other end of the pipette is connected with a rubber tube. The substance is introduced into the tissue of the tadpole by blowing into the tube, considerable effort being required in order to force the substance through the glass capillary. It is essential, while blowing, to gradually withdraw the glass pipette from under the skin.

Since large amounts, as well as a few particles of various substances were introduced, medium and large-sized tadpoles were chosen for the experiment. A successful injection could be easily determined, the material injected appearing under the skin as an opaque streak. Though in some cases the substances were introduced in excessive amounts (the strand of injected substance appearing to be over 3 mm. in length and to occupy half of the thickness of the fin) the animals did not show uneasiness from the effect of the operation. Hemorrhages could not be avoided during injection, but those specimens in which hemorrhage could be macroscopically discerned were eliminated. Observations in vivo were occasionally made and a large number of fixed tadpoles at different stages after injection were secured. The animals were allowed to live from 2 hours to 3 or 4 weeks after the injection.

The first set of experiments, of which a report is given in the present paper, consisted in injecting various amounts of edestin. This substance, insoluble in water, appears macroscopically in the form of minute particles, irregular in shape, rather uniform and rarely exceeding in size the eosinophilic granules of leucocytes. Every edestin granule ingested by cells can easily be identified in an eozin-azur preparation after Zenker-formol fixation, the granules staining a brilliant red.

We wish to express to Professor Gies our great indebtedness for chemically purifying this substance.

Structure of the Dorsal Fin of a Tadpole Tail.—A short description of the structure of the edge of the tadpole tail is necessary in order to know what kind of cells will be in immediate contact with the substance introduced into it. It consists of a plate of loose mesenchyme covered on both its surfaces by epidermis. The epidermis contains three differently organized layers of cells, all of them exhibiting frequent mitoses. The superficial layer of cuboidal epithelial cells is covered by a thin cuticular border. The basal layer of epithelial cells consists of characteristic cuboidal and columnar cells containing sharply defined filaments. The middle layer of the epidermis consists of pigmented cells with peculiarly incurved nuclei. Chromatophores in the form of many branched cells are not infrequently found in this layer. The epidermis is lined by a heavy basal membrane. Numerous white fibers traverse the tissue between the two basal membranes.

The loose tissue of the plate contains scattered mesenchymal cells and vessels, a few strands of smooth muscle tissue and nerves. No wandering cells of any kind are normally present in this tissue. A continuous layer of mesenchymal cells is situated directly under the basal membrane of the epidermis. These cells, as well as those situated within the plate itself, are typical loose connective tissue cells with numerous long cytoplasmic processes, with oval nuclei usually containing well-defined nucleoli and minute particles of chromatin. Occasional mitoses are found among them. Chromatophores are few in this tissue, but are not infrequently found surrounding the vessels and accompanying even tiny vascular branches.

Results of the Experiments.

In describing and analyzing the various phases of the reaction developed around the injected material, a distinction has to be made between the processes due to the injury proper and those which develop in consequence of the presence of foreign matter, digestible particulate protein in this case. The reaction due to the injury is exhibited partly by local cells, partly by elements brought in by the blood current from remote parts of the organism. The reaction due to the injury is, in these experiments, only slight in both of these aspects and is greatly overshadowed, shortly after the injection, by processes depending upon the presence of the injected material.

Effect Produced by the Injury during Injection.—Besides a slight injury of the epidermis, a few cells of which are sometimes carried into the mesenchymal plate, the direct consequence of the injection is the boring of a canal in the midst of the loose mesenchymal tissue and the filling of it by finely pulverized proteinic matter. It is remarkable how seldom appreciable hemorrhages are produced, and only very few blood corpuscles are regularly found free in the intercellular spaces as a result of the puncture of a few small vessels. By pushing the glass capillary under the epidermis the tissue is simply compressed, this compression being further exercised by the thick suspension of edestin after the withdrawal of the glass capillary. Microscopically the injected material appears in the form of a rather sharply defined strand of densely aggregated particles. The immediately adjacent tissue becomes slightly edematous and the white fibers traversing the fin plate swollen. Only few mesenchymal cells are found in direct contact with the edestin particles fifteen minutes after the injection. but even at that time these cells, if compared with those situated at a distance from the injected material, seem to be larger, their processes shorter and plumper and their cytoplasm often filled with tiny vacuoles giving it a foamy appearance. There is no doubt but that these changes in the mesenchymal cells, observed almost immediately after the injection, are not to be regarded as a specific response to the introduction of the edestin. Practically the same changes are seen after an injury produced by the introduction of the glass pipette without injection.

A breaking off of the syncytial arrangement of the cells and formation of typical wandering cells can be observed in those few mesenchymal cells which are in close proximity to the injured tissue. The number of wandering cells formed in loco at the expense of the mesenchymal cells is small indeed, the mesenchymal cells themselves being scarce. These local changes are slow and are quickly overshadowed by the appearance of cellular elements brought in by the blood stream.

Effect Produced by the Presence of the Injected Substance.— The changes observed in the region in which the injected masses are situated and dependent upon their presence are in part identical to those found, if only injury with the glass pipette were produced and the injection omitted. They differ greatly, however, in their intensity and in their duration. In describing the changes observed around and within the injected mass, three different phases will be reported under separate sections. (1) Appearance of wandering cells. (2) Digestive processes. (3) Phagocytes after digestion.

1. *Appearance of Wandering Cells.*—As mentioned above, vessels are seldom ruptured during injection and only a few groups of extravasated blood cells are found around the injected material. Among those the white blood corpuscles are seen to exhibit an intensive activity, the erythrocytes, however, lie inert in the intercellular spaces at first. Shortly after the injection the white blood corpuscles become greatly increased in numbers around the injected masses. Not only in nearest proximity but also at a cer-

tain distance there appear a great number of ameboid cells. The granular leucocytes are the earliest to come and during the first hours after injection they are found to be the most numerous. Their structure allows of an easy identification, their polymorphic nucleus being especially characteristic. Most of the leucocytes belong to the neutrophil class. Present in large numbers within the vessels, they are first to emigrate and to appear around the injected masses. Within the vessels and in close proximity to them they present the usual structure, but soon undergo considerable changes in their appearance. Their nuclei are often drawn lengthwise and appear in a thread-like shape. The chromatin in the nucleus is also arranged in the form of rod-like particles. This is of course due to intensive streaming movements exhibited by these cells. But a more substantial change takes place in their cytoplasm. This greatly increases in volume and though still granular is much less distinctly so.

In addition to the granular leucocytes, a number of ameboid cells with round nuclei appear soon. Twelve to fourteen hours after the injection they greatly increase in number and after twenty-four hours they are by far the most numerous among the cells infiltrating the region. These ameboid cells, which become most active in the process of digestion of the injected protein, exhibit the structure characteristic of the small lymphocytes. It is only after a thorough investigation that this conclusion has been reached. A few hours after injection when numerous polymorphonuclear leucocytes have already emigrated, the vessels throughout the tadpole and especially those in proximity to the injection begin to show an increasing number of small lymphocytes. The characteristic structure, which enables an easy recognition of these cells within the vessels, is also present even to minute details in cells which have recently emigrated and are situated near the injected substance. Small in size, they have a round nucleus containing numerous well-defined chromatin particles and no nucleoli. The nuclear membrane is chromatic and sharply defined. The cytoplasm is slightly basophilic and appears in the form of a narrow rim. Outside the vessels they exhibit numerous ameboid processes.

As these cells advance toward the injected mass they exhibit a series of changes, which make them rapidly acquire an entirely different structure and unroll a digestive activity little expected from the small lymphocytes. A rapid transformation of a small lymphocyte into a histiotopic wandering cell thereby takes place. Analogous changes have been observed in small lymphocytes, while in grafts of adult splenic tissue, the small lymphocytes wander out from the grafted tissue into the intercellular spaces of the allantois. Both nucleus and cytoplasm of these cells increase in size. The cytoplasm does not become more basophilic, as usually is the case with the hypertrophying mesenchymal cells. It stains light blue with Azur—II., and is often pervaded with minute vacuoles. The nucleus grows also; its chromatin, especially in the early stages, appears as in small lymphocytes in the form of irregular, rather heavy particles, but is soon converted into tiny fragments. The cells now differ entirely from the small lymphocytes in morphological appearance and wherever found in contact with edestin granules, rapidly ingest them.

The small lymphocytes are scarce in the circulation of a normal tadpole and the appearance of a great number of these cells, not only around, but in later stages within the injected mass, is at first glance rather difficult to account for. A study of the organs of the tadpole at this stage has revealed that the chief hemopoietic center is situated in the kidney. The blood-forming processes here exhibit a peculiarity not yet described in any hemopoietic organs of other embryos. The young blood stem cells, in the form of the lymphoid hemoblasts (large cell, basophilic cytoplasm, clear, round nucleus with a well-formed nucleolus) are here extremely scarce. Lymphoid cells are present in great numbers, but they exhibit the structure of small lymphocytes. The reciprocal relations of the blood cells in the kidney of the tadpole cannot be discussed at present, but it may be pointed out that the same characteristic feature was observed by one of us in the case of the axolotl years ago while studying the hemopoiesis in the perihepatic tissue in this animal.

The vessels in the kidney of the tadpole under experiment are always found to contain numerous small lymphocytes and an intensive proliferation of these cells is observed in the hemopoietic tissue of this organ. No decrease of small lymphocytes is brought about by the excessive withdrawal of these elements, but, on the contrary, the lymphatic elements seem to become even more numerous and mitoses particularly frequent.

The shifting of numerous small lymphocytes to the injected region produces evidently a stimulating effect upon the hemopoietic tissue of the kidney, similarly to a bleeding of the animal. In this case however the white blood corpuscles only are carried away in greater numbers and those remaining proliferate more intensively. A proliferation of small lymphocytes in the lymphatic tissue has been found under various conditions, after administration of small doses of X-ray in particular. A direct stimulating effect upon the lymphatic tissue was attributed to the action of the X-rays. It is, however, questionable whether this stimulating effect might not prove to be a secondary phenomenon due either to destruction or to an intensive shifting of the small lymphocytes from the region of their origin.

A third category of wandering cells may be recognized around the injected material. They are mobilized mesenchymal cells, in the early stages very few in number and never numerous. They are large, have a rather basophilic cytoplasm, and their nuclei frequently exhibit nucleoli. They have been already mentioned and their first appearance was ascribed rather to the direct effect of the injury than to the presence of injected masses. At the end of the first day after injection a curious activity is observable in the mesenchymal cells which form under the basal membrane a practically uninterrupted layer. These cells, normally flattened against the surface of the basal membrane and in sections appearing fusiform in shape, are now seen to separate from the basal membrane and to protrude their processes in the direction of the injected material. They leave their original places and are found among other wandering cells. Easily recognizable at first they gradually undergo a series of changes in the same direction as described for the small lymphocytes, and at a later stage these cells are no longer recognizable as such.

In order to make a complete picture of the wandering cells in the region of the injection, eosinophilic leucocytes should be mentioned, but they are very scarce. An endothelial origin of some of the wandering cells cannot be excluded altogether since occasionally small vessels are injured and their endothelial cells might be transformed into wandering cells. Their number, however, must be of small account indeed in comparison with those brought in by the blood stream.

2. *Digestion of Injected Edestin.*—The process of digestion is inseparably connected in our mind with a specialized system, all of which is derived from the entoderm. This includes a special cavity, in which the process is completed outside of cells and tissues. A group of specifically differentiated secreting organs are furnishing the enzymes, which, though of utmost importance in digestion, still escape a more precise definition of their chemical structure. We still judge of the presence of enzymes by the result produced by them. Owing to the observation of results obtained in the unicellular organism, we easily accord to the protozoa an intracellular power of digestion. In this case the digestive ferments become active within a vacuole surrounded by living cytoplasm, and while the conditions within this vacuole bring about digestion of the ingested food (whether living or dead) they do not affect in the same manner the cytoplasm of the acting organism.

It was Metchnikoff's merit to have pointed out in the multicellular organism the rôle of phagocytosis with subsequent digestion of the ingested material. The phagocytes described by him belonged to two classes distinctly and differently organized, the polymorphonuclear leucocytes and the macrophages. The polymorphonuclear leucocytes did not require much interpretation, but the macrophages became an object of numerous investigations. Their nature and origin are much disputed, possibly because results obtained by study of definite cases and true, if limited to these, were extended beyond the sphere of their control. The macrophages are derived in turn from mesenchymal and from endothelial cells. Blood stem cells (lymphoid hemoblasts) were also seen to become macrophages. A special line of resting or histiotopic wandering cells, eminently phagocytic, was observed to develop from the mesenchyme in later embryonic stages. The "cellules rhagiocrines" of Renaut and the clasmatocytes of Ranvier are endowed with phagocytic power. The polyblasts of Maximoff, derived from the small lymphocytes, are very active in inflammation processes.

No efforts have been spared to find structural peculiarities characterizing the mononuclear macrophages to the exclusion of lymphocytes. An ingenious method of identifying the macrophages was devised by H. Evans and elaborated by M. Simpson by staining a characteristic set of granules numerous in the mononuclear cells and very scarce in the lymphocytes.

That the class of macrophages, whether or not the mononuclear leucocyte of the blood stream belongs to it, are well-defined structural units, is not a disputed fact, nor is their phagocytic activity doubted. Their origin in the venous sinuses of the spleen, lymph nodes and bone marrow, in the region where endothelium and mesenchyme gradually merge into each other has been admitted. But do these well-established facts make the existence of a much more extended digestive activity in mesenchyme and its derivatives incredible in a multicellular organism? And why should digestive activity be necessarily limited to macrophages of serous cavities and to other cells identical with them as to their structure and origin?

A study of the conditions as they develop gradually in the embryonic organism will allow of an easy realization of the fact, that digestive activity is an inherent attribute of every cell, and that it is retained by that part of the mesoderm which remains the least differentiated in the form of mesenchyme. True that in vertebrates the ectodermal and mesodermal layers are soon separated from the yolk by the entoderm which remains in contact with the yolk and develops into a series of highly specialized digestive organs. But it is equally true that in earlier stages of development the cells of the primitive streak are in direct contact with the yolk and that the cytoplasm of all of them invariably contain yolk granules, which gradually disappear by intracellular digestion. All of the mesodermic phagocytes would derive their digestive power from the cells of the primitive streak, being their direct descendents. Actively manifested by the cells of the mesodermal anlage, the intracellular digestive power seems to be lost by a great number of highly differentiated mesodermic structures but is being retained by those mesenchymal elements, which, scattered through the whole organism, remain practically undifferentiated.

The conditions are by far more striking in those classes of animals whose eggs, as in the case of the tadpole, are rich in yolk, but segment completely. Though segmentation results in this case in uneven distribution of yolk among the various groups of cells, nevertheless all of them contain nutritive material and gradually digest it. The mesodermal cells are in this case developed in intimate association with the primitive entoderm, at a time part of it. The primitive blood cells are loaded with yolk granules gradually digested and used up. Later, however, the mesenchyme and its derivatives not being in contact with undigested protein, digestive capacity is not exercised by these tissues. But phagocytic digestive power remains inherent in them and is revealed every time mesenchyme or its derivatives are in the presence of particulate protein.

As seen from the preceding section, the injected mass is surrounded, six hours after injection, by a great number of wandering cells (Fig. 1) of different nature. Some of them are actually seen within the injected mass. The polymorphonuclear leucocytes are not only first to appear around the injected edestin, but they also are the first to bore their way into the rather compact mass of edestin. Wherever a group of edestin particles has been carried by the injection deeper into the tissues and found detached from the more compact mass, the granular leucocytes gather around them and often at this early stage edestin particles are found already ingested by them. While actively ingesting large amounts of edestin particles, the granular leucocytes may attain a considerable size. Their cytoplasm is often seen to be reduced to a narrow rim holding a large amount of ingested material. Its granular structure, becoming less distinctive, while the cell is moving toward the injected mass, has now practically disappeared altogether. But the nucleus retains its polymorphous structure and this makes the identification of these cells always easy. It is remarkable that a great number of polymorphonuclear leucocytes, found in the midst of the injected mass, are manifesting evident signs of degeneration. A chromatolysis is frequently observed in their nuclei at an early stage and later on these cells, even while containing in their cytoplasm edestin granules, are themselves subject to phagocytosis by other cells.

By far the most important rôle in the actual digestion of the injected mass belongs to those cells which are brought in by the blood stream in the form of small lymphocytes. We do not mean to say that the small lymphocytes as such devour a great amount of edestin and digest it. But there is no doubt that cells, the structure of which is identical to that of the small lymphocytes, emigrate from the blood vessels, approach the injected mass and, while approaching it, promptly change their morphological features. Such cells, lymphoid phagocytes as we will call them, if in contact with the injected mass of edestin, are seen, at the stage of six hours after injection, to contain edestin particles in their cytoplasm. The surface of their cytoplasm becomes indented and tiny cytoplasmic processes are seen to protrude between the injected edestin particles, to entirely surround them, to incorporate them into larger vacuoles and to repeat the same process again and again. Sometimes larger groups of edestin particles are seen to be surrounded simultaneously by larger cytoplasmic processes and incorporated into a common vacuole.

A greater and greater number of cells invade the edestin mass until a stage is reached, about 24 hours after injection, in which all of the edestin granules are found within the cells. At this time (Fig. 2) the conditions within the injected mass are difficult to analyze. The finest celloidin sections give rather obscure pictures. All the edestin granules are found in larger or smaller spaces surrounded by cytoplasmic strands, but the boundaries of the individual cells are indistinct. Cytoplasm, a little more abundant around the nucleus and easily recognizable as a part of a definite cell, is seen to merge gradually into cytoplasmic strands of neighboring cells. The whole region appears in the form of vast plasmodia, here and there interrupted by more or less definite fissures (Fig. 2).

Figure 3 shows the development of these conditions in the peripheral part of the injected mass. A vessel containing erythrocytes and a number of small lymphocytes is found to be in close apposition to it. A group of small lymphocytes in its proximity are seen with large ameboid processes. At this stage most of the edestin granules are incorporated in the cytoplasm of the lymphoid phagocytes, only a few groups still remaining outside. Most of them are situated in larger or smaller vacuoles. Apparently one cell may possess a number of such vacuoles. Though in general the cells appear to be well separated from one another, there may also be observed groups of cells which seem to have flown together.

As early as 16–24 hours after injection a change takes place in the numerical relation between the polymorphonuclear leucocytes and the lymphoid phagocytes, now both appearing in the form of large cells with vast vacuoles containing large amounts of edestin granules. The polymorphonuclear leucocytes, even more abundant than the lymphoid phagocytes early after the injection become now scarce and rather difficult to identify. A granular leucocyte, as admitted, is a highly specialized cell, stable and no longer capable of developmental changes. Would such a cell under experimental conditions be still capable of modifying its metabolism so as to manifest a fundamental change in its structure? This idea, incompatible as it seems with our knowledge of histogenesis of blood cells, is forced upon one's mind by the rapidity of the change in the numerical relation of these two different kinds of phagocytes. And only a detailed analysis of the conditions obtaining within the injected mass permits of a final refutation of this idea. The polymorphonuclear leucocytes numerous in early stages become less and less so, because of the association of various factors all working in the same direction. Soon after the first wave of emigration brings around the injected mass a large group of polymorphonuclear leucocytes, lymphocytic cells become more and more abundant within the blood stream and are seen to emigrate in larger and larger numbers. At the same time the polymorphonuclear leucocytes, well within the edestin mass, begin to degenerate in great numbers. In later stages many of them are seen to be ingested by the lymphoid phagocytes. As a result of the simultaneous association of these conditions, 1 or 2 days after injection almost all of the cells in the area of injection are represented by lymphoid phagocytes.

There is no doubt that while the process of ingesting the edestin takes place, actual digestion begins at about the same time. The digestion in this case is intracellular and similar to that observed in the protozoa. The edestin particles, found in rather large vacuoles, are gradually disappearing, and, on the basis of this observation, the conclusion must be reached, that enzymes are liberated by these cells into vacuoles in conditions permitting of digestion of the protein particles and not affecting the cytoplasm surrounding the vacuole. A rather interesting detail is observed in the digestive activity of the phagocyte. The edestin granules are frequently seen to be surrounded individually by thin strands of cytoplasm. Early after injection, the phagocytes are invariably seen to contain large groups of edestin granules in single vacuoles, but in later stages these groups are seen to be gradually separated by cytoplasmic strands (Fig. 4, four days) until every granule is surrounded separately. Whether, however, this step is necessary is difficult to tell.

While ingestion of edestin by the phagocytes has resulted in the formation of vast indivisible plasmodia (Fig. 2), the digestion and resorption of the edestin leads again to a definition of separate cell units. Two days after injection the region appears very similar to the condition found in the periphery of the injected mass at an early stage (Fig. 3). The phagocytes appear again individually separated and in denser regions only a few plasmodia are observed. This separation is completed 3 or 4 days after injection, as Fig. 4 shows. At this time (4 days after injection) the digestion in most of the cells has been completed. Some of them still show a large vacuole containing a number of edestin granules, but such cells have become scarcer. Other phagocytes contain two or three small vacuoles with a limited number or even with single granules. Cells are observed, also, which do not contain more than 2 or 3 small granules and finally a number of cells are found without any. Digestion in these cells has been apparently completed.

The digestive power is being exercised by the lymphoid phagocytes in a most conspicuous way. It is questionable, however, whether or not polymorphonuclear leucocytes actually digest the edestin particles found rather numerous in their cytoplasm at earlier stages. Mention was made above regarding degenerative changes frequently observed in the leucocytes within the injected mass. But even those leucocytes which persist at a stage when a great part of the edestin has been digested do not seem to prosper. They are now frequently seen ingested by the lymphoid phagocytes and undergoing a digestion within them. In Fig. 4 three lymphoid phagocytes are seen to contain polymorphonuclear leucocytes in their cytoplasm; the latter, themselves, had acted as phagocytes, for numerous edestin granules are still present in their cytoplasm. They succumb, however, now to the phagocytic activity of lymphoid phagocytes.

The structure of the lymphoid phagocytes is so characteristic as to permit an unmistakable judgment regarding their past activity and origin. And the new phase of their digestive activity which now is directed against the polymorphonuclear leucocytes is of great interest. Both lymphoid phagocytes and polymorphonuclear leucocytes develop in the same hemopoietic center and from the same stem cells. Both were brought in by the blood current to the region of the injection; they both moved into the injected mass and began their active ingestion of the edestin particles. But while ingestion by lymphoid phagocytes is followed by an intensive digestive activity, the polymorphonuclear leucocytes, though containing ingested edestin, seem to remain inert. The exercise of digestive activity by the lymphoid phagocytes seem to have stimulated and sharpened their digestive power and they begin to display it against cells of their own kind, the granular leucocytes.

The digestion of the injected mass of edestin is completed in about 6 or 7 days. There is no more trace of edestin granules in the tadpole tail at this time and the whole region, in which the edestin appeared as a compact strand of a 0.3–0.5 mm. in thickness, has been reduced to a line hardly perceptible to the naked eye and no more than 100 μ thick. Not only has the whole mass of injected edestin disappeared from this region, but the millions of cells which after 2 days of injection invaded it have left it. There is no indication whatever of degenerative processes in the lymphoid phagocytes. Their changes and fate will be discussed in the following paragraph.

3. *Phagocytes after Digestion.*—A cursory glance and comparison of a small lymphocyte traversing within the blood current the region of injection (Figs. 4 and 5) with the phagocytes scattered through it, is sufficient to establish the difference between these two types of cells, as such. Nevertheless, as seen from the preceding sections, the phagocytes did develop from the small lymphocytes of the blood stream through a series of changes which the emigrated cells exhibited while approaching the injected mass. The exercise of digestive activity by the lymphoid phagocytes seems to have further fixed the acquired structural characters and most of the phagocytes are seen to retain their new structure. They now appear in the form of large cells with especially abundant cytoplasm. They are very similar to the usual types of phagocytes encountered in the loose connective tissue and described under many different names (resting or histiotopic wandering cells, clasmatocytes, macrophages, cellules rhagiocrines). Only the loose mesenchyme in the tadpole does not normally contain any wandering cells. The mesenchymal cells though observed to transform occasionally into wandering cells after injection, are extremely scarce. No mononuclear leucocytes are present in the blood current at that time. The only cells capable of further progressive development and differentiation in the tadpole blood are cells which exhibit the structure of small lymphocytes. They are seen indeed to give rise suddenly to numerous generations of phagocytes. These cells after digestion retain their newly acquired structure and are easily recognizable. They exercise their digestive power locally but do not remain in this region indefinitely. They are seen to increase in number while supplies of injected edestin last, but as soon as all of it is consumed, the immigration of new cells stops. The phagocytes remain in this region while they are actively digesting the injected edestin, but then begin to emigrate individually. By far the great-

est part of the phagocytes are seen to leave the region and settle in new places.

The phagocytes are seen to be very active in leaving the region of the injection as soon as they have completed the digestion of the ingested particles. The large aggregations of these cells are being dispersed, and 4 days after injection the egress of phagocytes is very intensive. At this time lymphoid phagocytes can be detected at a good distance from their previous location. Small groups of phagocytes are gathered directly under the epidermis. Two or three weeks after injection they can still be recognized as cell units with definite structure. What is even more remarkable is, that the cells are seen not infrequently to undergo mitosis.

Though on the basis of the present experiments a complete description of the distribution of the phagocytes cannot be given, there are undoubtful indications that their new whereabouts are not confined to the tadpole tail. The phagocytes, while moving from the region of injection, come not infrequently across vessels. Vessels have grown by this time also into the injected region, and numerous phagocytes are now seen to be directly applied against the thin-walled vessels. It is also possible to detect some of these phagocytes within the vessels. It is natural that such pictures are extremely rare, because the phagocytes, once entered into a vessel, are promptly carried away.

The uninterrupted egress of the phagocytes reduces the wide strands of densely accumulated phagocytes to a hardly visible scar (Fig. 5). The cells in it are of two kinds: Typical mesenchymal cells and typical histiotopic or resting wandering cells. The latter are the less numerous. As compared with Figure 4, Figure 5 shows that the egress of the phagocytes is chiefly responsible for the loosening of the tissue and for the shrinking of the previously wide strand of the densely infiltrated region.

CONCLUSIONS.

The introduction of a large amount of edestin into the mesenchymal plate of the tail of the tadpole produces a local and a general reaction in the organism. The local reaction consists in a response of mobile and mobilisable cells and results in a dense infiltration of the injected mass with consecutive ingestion and digestion of the injected mass. The injected suspension of edestin is a powerful chemotactic agent for both the granular leucocytes and small lymphocytic cells.

Secondarily a general reaction appears in the blood-forming tissue of the kidney. It consists in intensive proliferative processes which seem to be in relation with the egress of small lymphocytes from this tissue.

The lymphocytic cells emigrated from the vessels gather around the injected mass, hypertrophy and gradually transform into typical histiotopic wandering cells or lymphoid phagocytes. They ingest large amounts of edestin granules and exercise a digestive capacity. The digestive activity manifested by these cells seems both to stabilize the newly acquired structure of the phagocytes and to sharpen further their digestive power.

The granular leucocytes though active in ingesting the edestin granules do not seem to be capable of digesting them. These cells, especially those containing edestin particles in their cytoplasm, are seen to succumb finally to the digestive activity of the lymphatic phagocytes. There exist undoubtful indications that some of the phagocytes have derived from the mesenchymal cells, but their rôle cannot be easily determined in these experiments.

Two days after injection all the edestin granules are found within the phagocytes; seven days after injection all of the edestin has disappeared.

The digestive processes within the injected region not only result in the formation of a generation of new eminently phagocytic cells, but seem to reflect secondarily upon the whole organism. Innumerable cells of the type of small lymphocytes are not only transformed into lymphoid phagocytes, but these phagocytes are soon found at a considerable distance from the region of injection and part of them are probably distributed through the blood current to more distant parts of the organism.

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EXPLANATION OF FIGURES.

All the figures are camera lucida drawings made with the Zeiss apochromat oil immersion 2 mm., and with the compensatory ocular 8.

Plate I.

Fig. 1. Zone of injection 6 hours after experiment. Edge of the injected strand of edestin at the right side of the figure. A vessel at the left contains erythrocytes, numerous small lymphocytes and one neutrophil polymorphonuclear leucocyte. The emigrated small lymphocytes in the proximity of the vessel are small. They hypertrophy while approaching the injected edestin. A number of granular leucocytes and three lymphoid phagocytes have penetrated the mass of edestin and begin to ingest its particles.

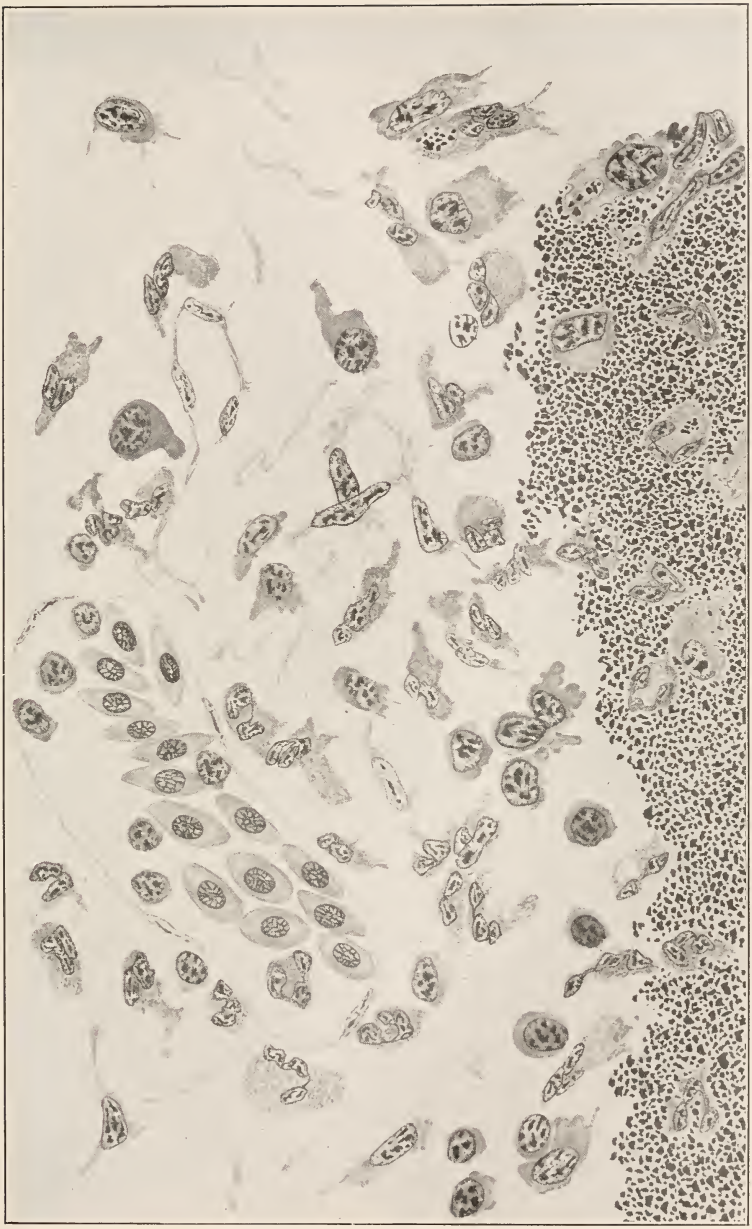

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PLATE II.

FIG. 2. Zone of injection 24 hours after operation. Central part of the large strand of ingested edestin. The cells form a uniform plasmodium. The edestin granules are seen to be separated into groups and surrounded by thin cytoplasmic processes.

FIG. 3. Zone of injection 24 hours after operation. Edge of the large strand of injected edestin. A vessel is seen to contain erythrocytes, small lymphocytes and a granular leucocyte. Most of the phagocytes contain large amounts of edestin and appear as separate cells, though a few of them have flown together.

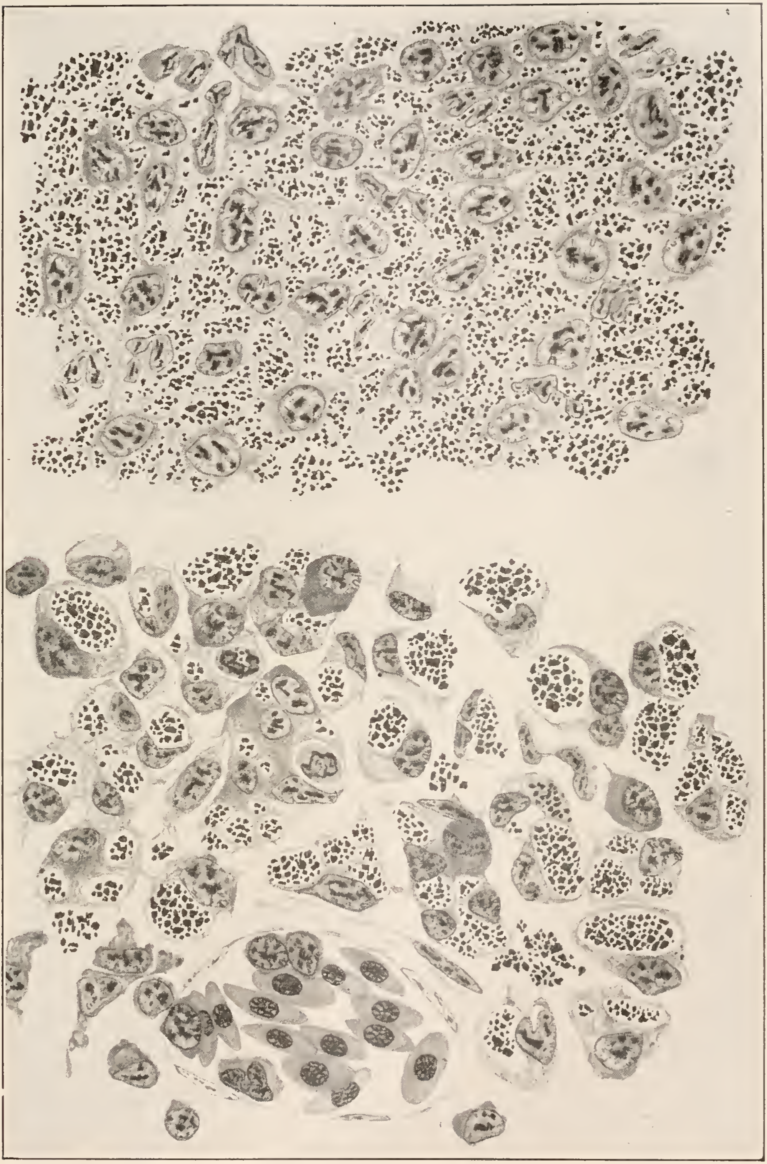

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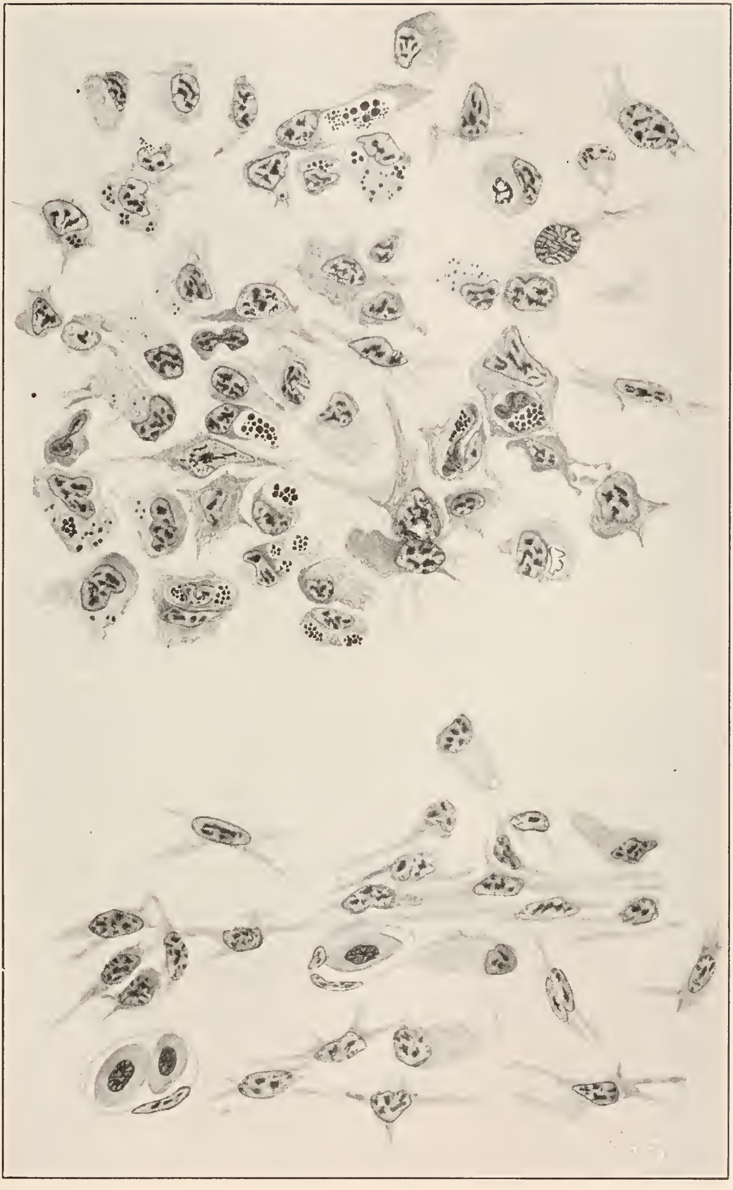
PLATE III.

FIG. 4. Zone of injection 4 days after experiment. A great number of phagocytes have completed the digestion of the ingested edestin; others contain still either small groups or even single particles of edestin. Three granular leucocytes with edestin in their cytoplasm are seen to have been ingested by the lymphoid phagocytes. The phagocytes become much less numerous than in the preceding stage.

FIG. 5. Zone of injection 7 days after experiment. A thin scar remains after completion of digestion of the injected edestin. It consists of mesenchymal cells, scarce histiotopic wandering cells (lymphoid phagocytes) and a few vessels traversing the zone.



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A STUDY OF INFLUENCES WHICH MAY AFFECT THE SEX-RATIO OF THE DEER-MOUSE (*PEROMYSCUS*).

FRANCIS B. SUMNER, MARY E. McDANIEL AND RALPH R. HUESTIS.

INTRODUCTION.

For the past eight years the senior author has been conducting breeding experiments upon California deer-mice, chiefly subspecies of *Peromyscus maniculatus*.[1] Throughout this period, fairly complete records have been kept of the births, with a view to the ultimate use of these data in a study like the present.[2] The work of tabulating the crude data and of computing the values herein presented has been chiefly performed by the junior authors. The senior author has, however, supervised the work throughout, and assumes responsibility for the accuracy of these various figures.

The data upon which this report is based are not in all respects as complete as might be desired for the study of sex-ratios, though we do not believe that their value is seriously affected by these limitations. Thus the number of individuals in a brood was frequently not determined until the expiration of some days, or even as much as two weeks after birth. As a rule, the brood was discovered on the day of its birth, or within one or two days thereafter. The number of young was commonly recorded at that time, in cases where it was possible to do so without seriously disturbing the mother. At latest, the number was recorded about 16 days after birth, at which time we have regarded it as safe to clean out the cage and change the nesting material.

Record of the number of each sex in a brood was not usually made at the time of this first count. In some cases it was made within the next few weeks; in others it was deferred until the time of marking, when the broods were broken up, those of different sex being segregated, and each individual being registered and given its proper serial number and identification mark. There has been no uniformity of practice in regard to the age at which the mice have been thus separated, marked and registered. Whenever practicable, this has been done within six or eight weeks after birth, but three months or more have sometimes elapsed before it has been convenient to do so. When, as is commonly true, no deaths have occurred during the interval which has elapsed since the first count, no harm can have resulted from thus deferring the record of sex.

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[1] Sumner, 1920, and papers therein cited.

[2] There are here included a few hundred mice from the records of Mr. Huestis, and a few hundred others from the records of Mr. H. H. Collins. We are indebted to Mr. Collins for permission to use these last.

A few words are worth while at this point in reference to the possible bearing which such imperfections in our records might be supposed to have upon the results set forth in this paper. As already stated, we do not believe that they seriously affect their value. In the first place, such errors as actually exist are ones of omission. The number recorded for certain broods is doubtless too small, owing to the occasional presence of stillborn young, or of ones which died within the few days following birth. On the other hand, we are confident that mistakes in the identification of the sexes have been so infrequent as to be negligible.

Regarding this matter of incompleteness of the entries, several things must be said. Firstly, it is doubtful whether any records are possible which are perfectly complete in this respect. It is a practical impossibility to inspect every brood immediately after birth, and in the interval which elapses the mother may eat such stillborn or feeble offspring as are present.

In the second place, the number of dead or defective members of a litter is small in *Peromyscus,* as compared, for example, with ordinary white mice. Stillborn young may be very common among the latter. In our experience they have certainly been rare among the former. As regards very early postnatal mortality, this is, from the nature of the case, difficult even to estimate from the data at our disposal. Reference to the death rate somewhat later in life may, however, be instructive. Of the 1,567 broods, comprising 5,050 young, which are listed in our records, only 305 broods, or less than 20 per cent., showed any mortality whatever between the time of the first count (16 days or less) and the time of marking (60 ± days). The number of young of unknown sex which died during this interval was 384 or about seven and a half per cent. of the total.

Thus it is not possible that a differential death rate (unless very marked) would seriously affect the sex ratio of the survivors. We have, however, fully considered the possible influence of such a differential death rate, and have endeavored to determine its degree, if actual. This has been done by comparing the sex ratios in broods in which deaths (of undetermined sex) are known to have occurred with broods in which no deaths are known to have occurred (see below). Unfortunately the figures are available for only a very small number of offspring of known sex (40) which died from natural causes before the "sexing" of the broods to which they belonged.

As already stated, the total number of broods recorded is 1,567, comprising 5,050 young, or an average of 3.22 mice per brood. According to sex, these were distributed as follows:

Males	2,295
Females	2,357
Sex undetermined (dead, killed and escaped)	398
Total	5,050

For those of known sex the sex ratio (number of males per hundred females) is 97.37 ± 1.93.[1]

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[1] In computing the probable errors we have employed a formula furnished us by Dr. Raymond Pearl, viz.:

$$\pm 67.45\,(1 + R)\sqrt{\frac{R}{n}},$$

in which R is the number of males divided by the number of females, and n the total number of individuals concerned.

Our colleague Dr. G. F. McEwen has computed a somewhat simpler formula for the sex ratio:

$$\pm .6745\sqrt{\frac{p\,q}{n}} \times 4,$$

in which p and q are the percentages of males and females respectively. This gives approximately the same values as Dr. Pearl's formula when the sex ratios do not depart widely from 100. Neither formula is accurate when the departures are very wide.

It must be stated that the probable errors employed in this paper are about

Since, as will be shown presently, there are rather wide seasonal differences in the sex ratios found by us, and since the different months are represented very unequally in our records, it is of interest to present the mean of these separate monthly ratios. This figure is 95.65.

Both of the foregoing figures are distinctly smaller than have been given by various writers for rats and mice,[1] as well as for man and some other animals.[2] In most cases a decided excess of males has been reported. Miss King (1918), for example, from the records of 2,818 white rats born in her "stock" (*i.e.,* unselected) series, obtained a sex ratio of 104.6.

Some attention should here be devoted to the possibility, already referred to, that the sex ratio which we have obtained for *Peromyscus* has been influenced by differential mortality. As is well known, the sex ratio among stillborn infants is very high, being frequently given as 130 or more;[3] and for cattle a similarly higher prenatal mortality among the males has been reported.[4] For *Peromyscus* we have no data on this subject since the sex of stillborn young was in no case determined. As regards early post-natal mortality, also, our direct evidence is very meager, so much so as to be almost worthless. 424 deaths occurred between the date of counting and the date of marking and registration. Owing to cannibalism and other causes, it was frequently impossible to determine the sex of these dead individuals, and in many other cases we neglected to do so; but this was done in 40 cases. Restricting our consideration to those mice which died during the first two months of life, we have 31 individuals, of which 20 were males and 11 females. From these figures one

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twice as great as those which would be obtained by another formula which has been widely followed (see Pearl and Pearl, 1908), and are therefore much safer as a basis for estimates of the significance of results. Indeed it turned out that certain highly interesting conclusions which we had drawn at the outset had to be relinquished on this account. It may be added that the conclusions of certain other writers are greatly weakened if the formula here employed is substituted.

[1] King, 1911, 1918.

[2] Morgan, 1907, pp. 365–366; 1913, pp. 230–231.

[3] Morgan, 1907, p. 368; Schultz, 1918, p. 264.

[4] Jewell, 1921.

might be led to suppose that the young males were subject to a mortality about twice as great as the females. But aside from the extremely limited numbers here concerned, there are other reasons for believing that no such marked differential mortality exists in this case.

Separate computations have been made for the broods in which no deaths were recorded and for the broods in which deaths are known to have occurred. Of the former there were 1,301 broods, containing 4,081 individuals, 2,020 being males and 2,061 females. The sex ratio here is 98.01 ± 2.07.

Of the broods which are known to have sustained losses prior to marking there were 297, originally comprising 1,079 individuals. The number dying (or missing) was 438, of which 398 were of unknown sex. The surviving 641 mice comprised 312 males and 329 females, giving a sex ratio of 94.83 ± 5.06. Thus we do find a slight difference between the complete and the depleted broods, though the difference is a quite non-significant one, statistically speaking. The reasonable inference is that the mortality, during the period here concerned, is approximately equal for the two sexes. In any case, there can be no such disparity in their respective death rates as the meager record of identified dead might lead one to suppose.

Seven possible influences, which have been held by various writers, to affect the sex ratios of animals, have been considered in our treatment of the data at hand. These are: (1) season, (2) size of the litter, (3) race, (4) hybridization, (5) inbreeding and outbreeding, (6) order of birth, (7) diet. As will appear in the ensuing pages, we have some evidence that the first, second and fourth of these influences are actually effective in the case of *Peromyscus,* though perhaps in no case can this evidence be regarded as wholly conclusive. As regards the other four possible influences, the evidence is inconclusive or is quite negative.

To the foregoing list of possible factors affecting the sex ratio we may add an eighth, namely the *year.* Surprising and inexplicable as the fact may be, we have found large and sometimes significant differences between the sex ratios for certain of the years covered by our observations. Indeed these differences are statistically more certain than any of the others which appear in our records. Such a relationship is not, of course, an ultimate fact, incapable of further analysis. But it does not seem to be dependent upon any of the other agencies for whose influence we have positive evidence, nor are we at present able to offer even a plausible explanation of it.

We shall consider, in turn, the supposed influences which have been enumerated above.

SEASON.

Before proceeding to discuss the possible influence of season upon the sex ratio of *Peromyscus,* it should be stated that the mice in question have, with a few exceptions, been born and reared under atmospheric conditions closely approaching those of the outside world. The building ("murarium") used for the purpose was specially constructed with a view to securing this result throughout the year.

Our studies of the relation between season and the sex ratio emphasize the danger of basing conclusions of this sort upon inadequate statistical data, even though the results may at first seem to be "significant," according to accepted standards. Before we included the records for the last two years (1920 and 1921), the evidence of a well-marked biennial rhythm in the sex ratio of *Peromyscus* seemed fairly conclusive. The seasonal cycle, when plotted graphically, was perfectly consistent, there being an uninterrupted rise and fall twice annually. Likewise the differences between the highest and the lowest ratios were of tolerably high "significance." The inclusion of the data for these two later years greatly weakens the evidence for a definite seasonal cycle in the sex ratio. But the possibility still seems to be great enough, and the facts, if true, of sufficient interest, to warrant our presenting the evidence rather briefly.

The following table gives the sex ratio for each month of the year, likewise (in parenthesis) the number of individuals upon which this ratio is based. The table is based upon the combined data for all of the years and for all the different series of mice.

January	(185)	88.78 ± 8.92
February	(287)	90.07 ± 7.04
March	(722)	106.29 ± 5.14
April	(343)	113.04 ± 8.24
May	(506)	94.62 ± 5.63
June	(405)	87.50 ± 5.80
July	(347)	91.71 ± 6.57
August	(565)	103.97 ± 5.84
September	(445)	96.04 ± 6.13
October	(406)	107.14 ± 7.17
November	(236)	78.79 ± 6.93
December	(205)	89.81 ± 8.37

These figures and the accompanying graph (Fig. 1) reveal the existence of two annual maxima, one occurring in March and April, the other extending from August to October. Alternating with these are summer and winter minima.

It will be seen that the differences between the successive months, taken by themselves, are of very doubtful significance, while even the difference between the highest and lowest months of the year (April and November) is only a little more than three times its probable error. With the exception of September, however, the seasonal cycle presents a perfectly consistent picture, there being an almost uninterrupted rise and fall twice annually.

The dotted line in the figure is based upon the date of conception, instead of the date of birth. The date of conception has been computed, for each brood, by subtracting 22 days from the date of birth, this being the usual period of gestation.[1] Such a procedure has inevitably resulted in the transference of part, though not all of the broods to the month immediately preceding. Were the period of gestation exactly one month, the one graph would be an exact duplication of the other. As it is, they present some obvious differences of form. The chief of these is the division of the fall "maximum" into two entirely distinct peaks.

Since, for various reasons, it does not seem probable that the differences between the consecutive months should be regarded as significant, we may profitably combine our monthly birth records into four seasons of three months each. Reference to the table of monthly ratios shows that we may distinguish two high periods

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[1] Sumner, 1916.

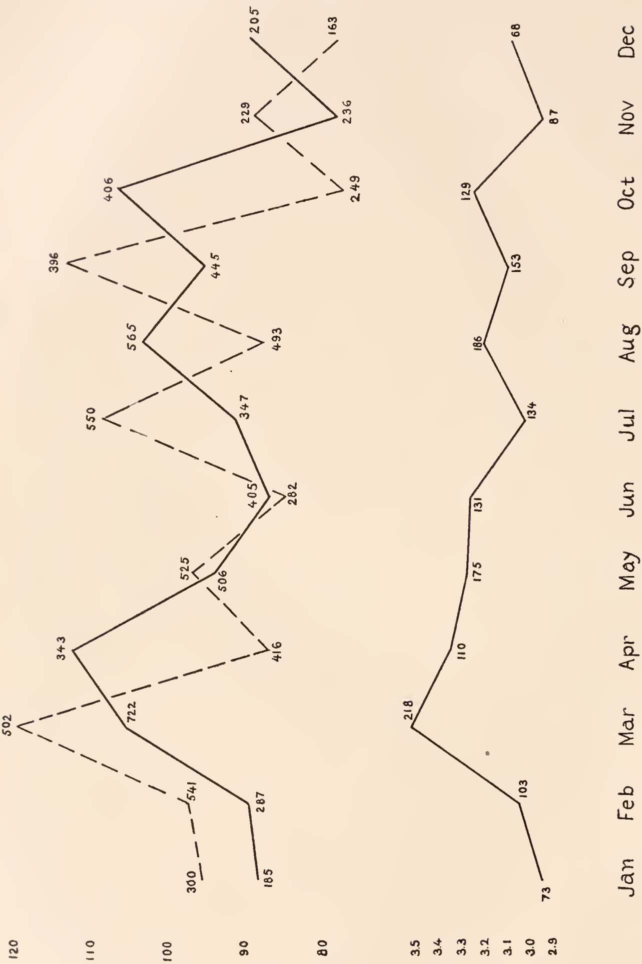

FIG. 1. Above: the sex ratio of *Peromyscus* for each month of the year, computed from all the material. The continuous line is based upon the date of birth, the broken line upon the date of conception. Ordinates indicate sex ratios (number of males per hundred females). The figures along the graphs denote the number of individuals born (or conceived) during each month of the year.

Below: mean size of brood for each month of the year. Figures along line = number of broods.

and two low periods annually. The sex ratios for these four periods are as follows:

(1)	February–April	104.23 ± 3.85
(2)	May–July	91.48 ± 3.48
(3)	August–October	102.29 ± 3.68
(4)	November–January	85.21 ± 4.62

The greatest difference between two of these ratios is that between the first and fourth periods. This is 19.02 ± 6.01. Taken by itself, such a difference is commonly regarded as having a probable significance.

When the same broods are grouped according to date of conception, the figures become:

(1)	January to March	105.35 ± 3.85
(2)	April to June	91.09 ± 3.50
(3)	July to September	102.68 ± 3.66
(4)	October to December	82.10 ± 4.38

Here, again, the greatest difference is between the first and fourth periods, being, in this case 23.25 ± 5.83, or almost exactly four times its probable error.

Figure 2 is based upon the sex ratios for the four 3-month periods of the year, both when the broods are grouped according to date of birth and according to date of conception. The two graphs are seen to be in very close agreement, though the divisions between the 3-month periods have, in the second case, been advanced by one month.

It is when we subdivide our material into groups of one sort or another that the inconstancy of these seasonal differences becomes evident. Dividing our broods according to years is not very instructive, owing to the relatively small number born in any one year, taken singly, and particularly to the exceedingly meager records for certain months. These graphs (not here reproduced) show various extreme fluctuations which must be regarded as purely accidental. Five[1] of the seven "curves" show, however, what may be regarded as spring and fall maxima, though the position of these varies somewhat from year to year. Two of them, on the contrary (including one of the fullest years) give little indication of such relations.

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[1] Those for 1915 to 1919 inclusive, 1920 and 1921 being the exceptions. No graph is possible for the first year (1914) owing to the small number of months represented in our records.

More instructive are the results of combining these years into three groups of two to three years each, and plotting the seasonal cycle from the combined data for each of these groups (Fig. 3). The resulting graphs require no further discussion.

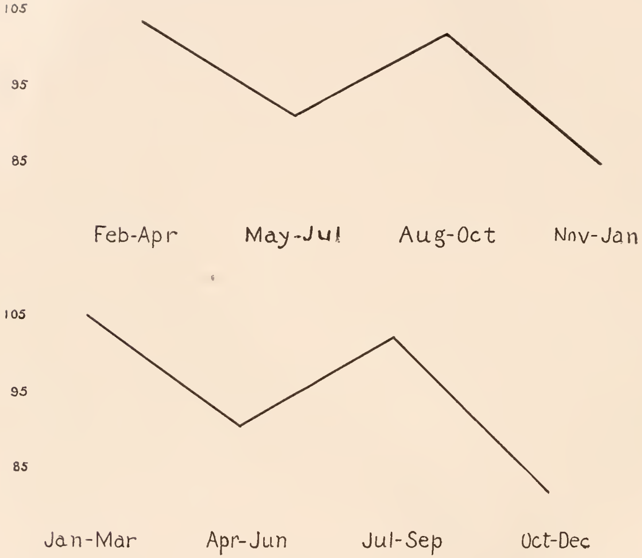

Fig. 2. Sex ratio graphs obtained by combining monthly records into "seasons" of three months each. The lower graph is based upon date of conception, the upper upon date of birth.

The data for the separate years were also grouped into 3-month periods, and graphs plotted for inspection (not reproduced). In 6 cases out of 7, there was a well-marked fall from the first to the second period, just as in the lot as a whole (Fig. 2). In 6 cases out of 7, likewise, there was a rise from the second to the third period. In only 3 cases out of 7, however, was there a fall from the third period to the fourth, one line remaining horizontal and the other three rising. (One of these last is based upon only 19 individuals.)

Two other methods of subdividing our material have been employed, these giving conflicting results. When we plot separately the hybrid mice and those of pure race (Fig. 4), we find that each of these groups displays a pretty well marked biennial rhythm. This is, however, much more pronounced in the former group than in the latter, and it is also to be noted that the "fall maximum" occurs in one case in August, in the other in October. In passing, let us point to the distinctly higher sex ratios shown by the hybrids throughout most of the year, a fact to which we shall refer again.

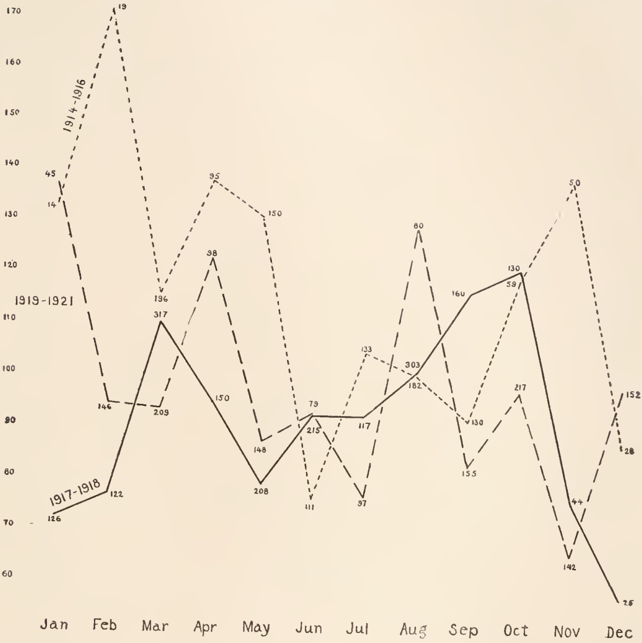

FIG. 3. Seasonal variations in the sex ratio, for three groups of years, treated separately. The continuous line (1917–1918) is based upon the largest number (1,917). Ordinates indicate sex ratios. Numbers along graphs indicate numbers born in each month. Certain of the larger fluctuations will be seen to be due to limited numbers of individuals (*e.g.*, in 1914–1916).

Our material was likewise subdivided according to the size of the brood, the seasonal cycles for broods of one to six individuals

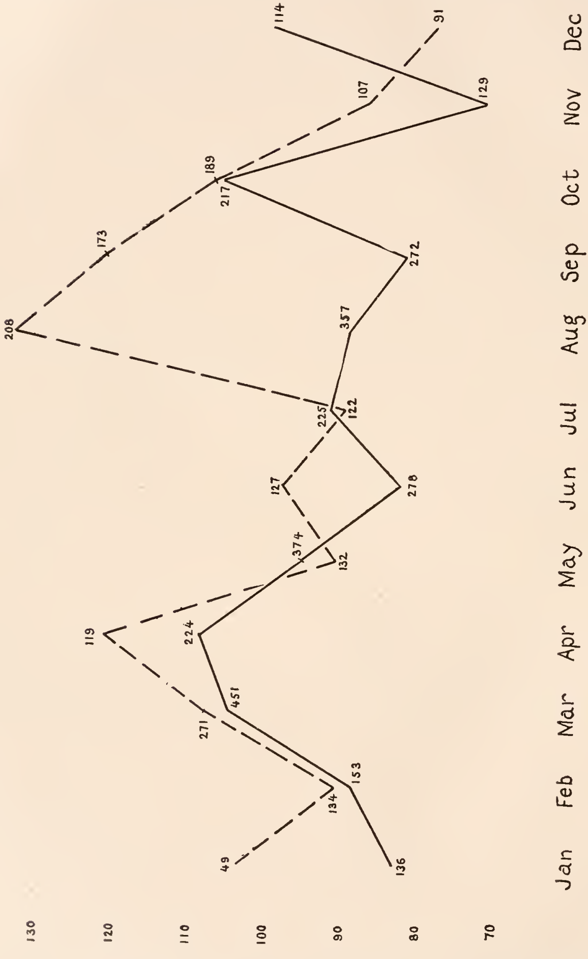

FIG. 4. Seasonal variations in the sex ratio for pure and hybrid stock, treated separately. Continuous line = pure. Broken line = hybrids.

respectively being plotted separately. Here again, the numbers comprised in some of these groups were so small that the graphs

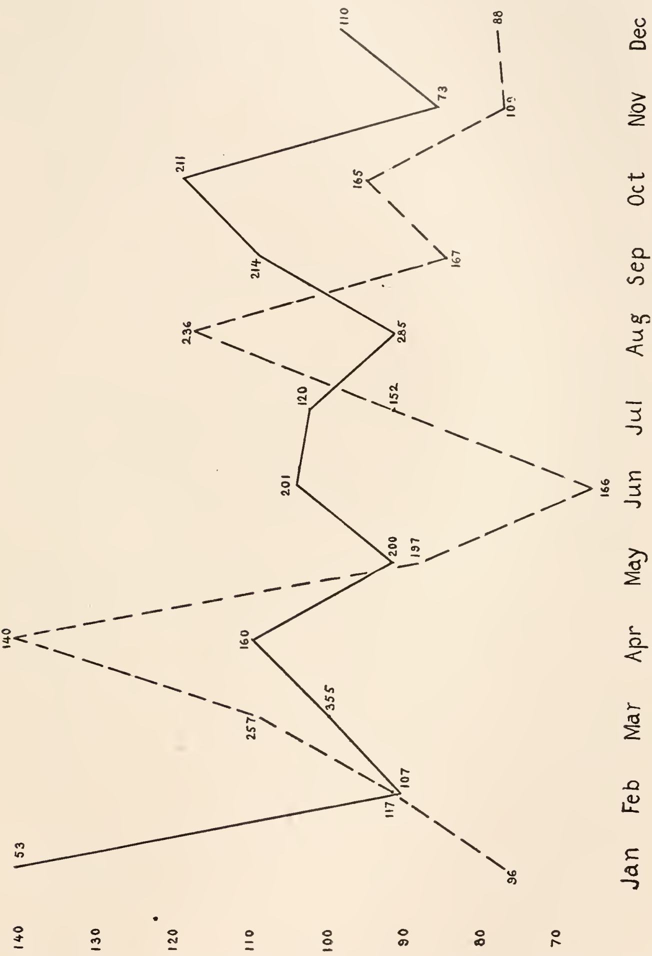

FIG. 5. Seasonal variations in the sex ratio, for small (1 to 3) and large (4 to 9) broods respectively. Continuous line = large. Broken line = small.

as a whole were rather confusing. We have, however, combined the data for small broods (one to three individuals) and for large broods (four to nine individuals) respectively (Fig. 5). This gives us two groups, each comprising about half of our material. The former group exhibits a very pronounced biennial rhythm with maxima in April and August.[1] The curve for the latter group, however, although based on somewhat greater numbers than the first, agrees only in having a well-marked fall maximum (September–October), with lower ratios in the summer and winter. There is but slight evidence of a spring maximum, and there are other irregularities in the "curve" for this large subdivision of our material. In general, none of these differences are of probable statistical significance. The high point in January cannot be regarded seriously, owing to the small number of individuals (53) for that month.

It must be conceded that the differences in the seasonal cycle, shown by these two groups of mice, seriously weaken our evidence for the existence of significant seasonal differences of any sort. For it does not seem likely that mice belonging to broods of different sizes actually behave differently in this respect.[2] It seems more likely that, for present purposes, we have two random samples of the population.

However, the fact stands that considerable differences have been found in the sex ratios of *Peromyscus* at different times of the year, whether or not these differences are due to "chance" (*i.e.,* errors of random sampling). Let us grant, for the moment, that the differences are not accidental. It then remains to consider whether they are due to season *per se*. It will be shown below that our hybrids as a class give considerably higher sex ratios than do the mice of pure strain. Also, quite independently of this last, it will be shown that mice born in different years differ widely in their sex ratios. The question arises: Can it be possible that the seasonal differences which we find are due either to the unequal distribution of hybrid and pure-bred mice throughout the twelve months, or to the unequal part which the mice of different years may have had in determining the various monthly ratios?

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[1] The difference between the figures for April and June is more than four times its probable error.

[2] Even this is not impossible, however, for there is some evidence (see below) that smaller and larger broods differ in their mean sex ratios.

The first of these possibilities is set aside by reference to Figure 4, showing the seasonal cycles of the pure and hybrid stocks plotted separately. The second we have tested by ascertaining the actual effect of each year's quota in determining the sex ratio for each of the twelve months.[1] But an examination of these figures (which it is not worth while to present here) does not support such an explanation of our seasonal cycle. Unless, therefore, all of these monthly differences are due to errors of random sampling, they are probably caused in some more or less direct way, by seasonal changes of temperature or some other physical agent.

The findings of certain other investigators regarding seasonal differences in the sex ratio may be appropriately considered here. King and Stotsenburg (1915) have presented data from 7,619 white rats reared at the Wistar Institute. The exact temperature conditions existing in the animal quarters are not discussed by the authors, though it is stated that the provisions for heat regulation were inadequate, and that the rats suffered greatly from heat during the summer months. It may be presumed that the rooms were heated to some extent during the winter.

When grouped by months, the entire data of King and Stotsenburg show what might be construed as a biennial rhythm. As regards the position of these annual maxima and minima, their results are in some respects in direct contradiction to ours. One well-defined maximum covers the period from June to August, another, the period from October to December. Minima occur in March and September. The second of these is of brief duration, being bounded on either side by months having high sex ratios. The former, on the other hand, is merely the lowest point in a prolonged period of low sex ratios.

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[1] A new set of monthly ratios was computed as follows: For each month, the number born in each of our years was multiplied by the general sex ratio for that year, and the sum of these products was divided by the total number born in that month. If the monthly differences which we have discussed above were due to the different seasonal distribution of the mice born in different years, this new set of monthly ratios ought to show much the same relations as the set computed earlier.

When plotted according to 3-month periods, the records of King and Stotsenburg show an annual minimum in the spring (March to May) and an annual maximum in the summer (June to August), while an intermediate condition is indicated for both fall and winter.[1] Our figures, on the other hand, show maxima in the spring and fall, minima in the summer and winter, though the "seasons" adopted by us commence a month earlier than those adopted by the former authors.

The marked differences found between the seasonal cycles of *Peromyscus* and the white rat might plausibly be attributed either to the difference of species or to differences in the environmental conditions under which the two sets of experiments were conducted. On the other hand, it appears to us that the reality of the seasonal cycle described by King and Stotsenburg is subject to exactly the same doubts as that described by us. The fact that the two groups of years into which the rat experiments were divided gave quite contradictory relations for the winter months certainly gives us reason for such skepticism.[2]

Heape (1907) gives evidence for the existence of seasonal differences in the sex ratios of dogs. The records for nearly 18,000 greyhounds show sex ratios which fluctuate irregularly between 111 and 128 during the months of January to September inclusive. In October, however, the curve rises to 145, in November to 180, and in December to 195. As regards the dates of conception, these last three months become August, September and October. For collies, on the contrary, he says that there is "no evidence that conception at any particular time of year affects the proportion of the sexes born." It must be added, however, that his figures for the collies indicate very considerable monthly differences among the sex ratios, and that some of these differences would seem to be of statistical significance.

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[1] The foregoing statements refer to the entire data of King and Stotsenburg. The material is divided by the authors into two groups which are partially discordant with one another. One of these (that for 1911–1913) gives a graph which is almost exactly the converse of ours.

[2] It is possible, however, that unknown influences (other than random sampling) caused differences in the sex ratio from one year to another. That such annual fluctuations actually occur in *Peromyscus* will be pointed out below.

For man, Heape (1909, 1909*a*) has compiled data, based upon census records of the births of more than 175,000 whites and negroes in Cuba. Unfortunately he does not give the number of births nor the sex ratios, for every month of the three-year period with which he deals, but only the figures for certain selected months of each year, namely, those showing the highest and lowest birth rates. We are therefore obliged to take Heape's conclusions to a large extent upon his own authority.

There are, he tells us (1909*a*) two seasons of high birth rate, a major one in July and August, and a less marked one in November and December. There are likewise two seasons of low birth rate, the chief of these being in January and February, the lesser one in September. During the periods of high birth rate, we are told, the sex ratios are relatively low (102.9 to 105.5 for whites; 93.3 to 104.1 for negroes). During the periods of low birth rate, on the other hand, the sex ratios are relatively high (106.2 to 113.0 for whites; 99.8 to 116.3 for negroes). Thus, there are "two sharply-defined breeding seasons each year . . . experienced by both whites and colored at the same time," one of these being more marked than the other.

These breeding seasons, Heape contends, are not related to the periods of the year at which marriages are most frequent. He seeks for correlations between certain meteorological conditions and the periods at which conceptions occur with greatest and with least frequency. The former periods would naturally fall in October–November and February–March, the latter in April–May and in December. In regard to these seasonal correlations, Heape states: "Reference to records of temperature, barometric pressure, humidity, etc., shows that these bursts of reproductive activity always take place at times when there is a marked change of climate; the one in the autumn . . ., the other in the early months of the year . . . it is obviously not a definite temperature, but the experience of a *change of temperature* which induces this boisterous generative activity" (1909, pp. 35, 36). Likewise, "my tables demonstrate that the greatest excess of females is produced at times of greatest fertility."

King and Stotsenburg likewise believe that there is a relation between breeding activity and the sex ratio. It is stated that "the rat breeds more readily in the spring than in any other season of the year, and there is a second, less pronounced, period of sexual activity in the early fall," while relatively fewer litters are produced in July and August. Their graphs show low sex ratios in the spring, followed by high ones in the summer, and low ones again in the fall. As already stated, the results for winter are contradictory with one another.

Our records for *Peromyscus* are unfortunately not adapted to revealing definite periods of increased or diminished reproductive activity, since the matings were to a large extent controlled in accordance with the demands of the breeding experiments.[1] But it is to be noted that the seasonal fluctuations which we have found in the sex ratio of *Peromyscus* correspond as little with those reported by Heape as they do with those reported by King and Stotsenburg. The relations which Heape believes to be shown by the Cuban statistics, even if applicable to man in that particular locality, cannot be generalized for all mammals nor for all places.

It is worth remarking at this point that the differences just discussed, between our findings and those of certain other writers, are typical of the conflicting results which pervade the entire literature of sex determination.

SIZE OF THE BROODS.

The 1,567 broods here recorded ranged in size from 1 to 9, though we have only nine records of broods containing over 6 young (seven of 7, one of 8, and one of 9). The mean size of all these broods was 3.22. This figure naturally varied somewhat according to the year (2.57 to 3.67), the season (2.96 to 3.53), and the race (2.76 to 3.78), etc.

The size of the litter will first be considered in relation to its possible influence upon the sex ratio. This part of our discussion need not be long, since the correlation in question is decidedly doubtful. The following table gives the sex ratios for mice belonging to broods containing from one to 7 (+) individuals respectively, excluding broods in which individuals of unknown sex are known to have died, and ones of probably mixed parentage (dual maternity).

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[1] The numbers born in November, December and January are well below those born in any other months of the year, and it is likely that this fact is due, in part, to an actual slowing down of reproduction, at least under the conditions of our experiments. But it is questionable whether any other seasonal differences in fecundity can be fairly inferred from our records.

Number in Brood.	Males.	Females.	Ratio.
1	64	61	104.9 ± 12.7
2	232	252	92.1 ± 5.6
3	624	657	95.0 ± 3.6
4	677	667	101.5 ± 3.7
5	264	256	103.1 ± 6.1
6	88	92	95.6 ± 9.6
7–9	28	17	164.7 ± 33.8

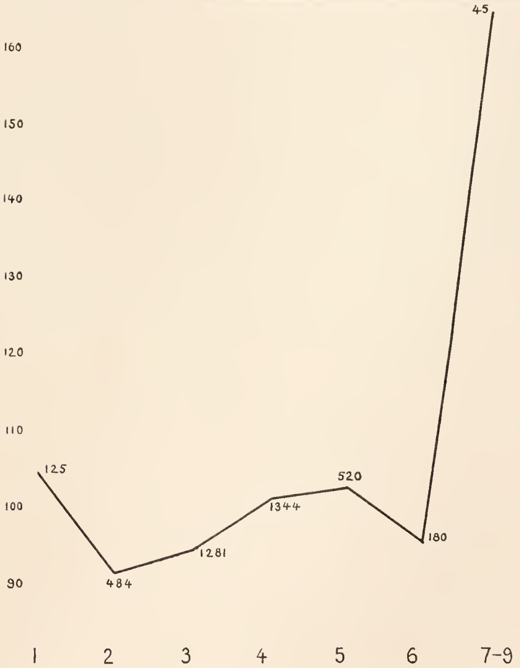

FIG. 6. Variations in the mean sex ratio, according to the size of the brood.

It will be seen (Fig. 6) that the sex ratio for broods containing from 2 to 5 individuals, inclusive, are arranged in a regularly ascending series, while the last group (7 and over) gives us a ratio far in excess of any of the others. Exceptions to this general trend are groups 1 and 6, though it will be noted that these two (as well as the last) are based upon relatively small numbers.

Despite the absolute magnitude of some of these differences, however, their statistical significance is in every case very doubtful. In most instances they are less than twice their own probable errors. When we combine broods of 1 to 3 and 4 to 9, inclusive, the sex ratios of these major size groups become 94.85 ± 2.94 and 102.42 ± 3.01, respectively. The difference between these values is rather less than twice its probable error. Here, then, as in the case of season, we cannot say with any confidence that the size of the brood is a factor which influences the sex ratio.

It might be inferred, moreover, that this correlation, even if actual, results from the fact that the smaller broods are ones which have been depleted by unrecorded deaths, and that the death rate has here been higher among the males. This explanation derives some support from the fact (p. 128) that among the very small number of identified dead included in our records, the males greatly preponderated. It is rendered improbable, however, by the fact (p. 129) that broods which are known to have been depleted give a sex ratio which is not significantly lower than that shown by the broods in which no deaths are known to have occurred.

Nor do our records afford any ground for the belief that these differences in the sex ratios of broods of different size depend upon the unequal distribution of such broods throughout the year. And it is equally improbable that the seasonal cycle, discussed above, is dependent upon the seasonal distribution of large and small broods. It happens that the seasonal cycle, in respect to the mean size of the brood, does correspond pretty closely, on the whole, with the seasonal cycle for sex ratio (Fig. 1). But the differences, in the former case, are so slight that they could not at most account for more than a fraction of the differences in the latter.

King and Stotsenburg obtained conflicting results from two large subdivisions of their material, when arranged to test the possible correlation between sex ratio and size of litter. The authors cautiously conclude that "the lack of uniformity in the results of this arrangement of data indicate that apparently there is no well-defined relation between litter size and sex in the albino rat."

Weldon (1907), on the other hand, believed that he had obtained some evidence that a positive correlation existed between the size of the litter and the sex ratio in mice. This relation was discernable only when the mean size of litter in different generations was considered, not being evident in respect to individual litters within a generation. The figures presented are not, however, very impressive.

Combinations of the Sexes in Individual Broods.—Thus far, the relative numbers of the sexes in broods of different size have been dealt with by methods of mass statistics. The total for each sex has been computed, and the ratio between these totals obtained. Such treatment would entirely obscure one possible phenomenon of high interest, namely the tendency of members of a litter to agree with one another in respect to sex. Do we, for example, encounter broods consisting of four or five of the same sex more frequently than would result from chance?

This question we have endeavored to answer by arranging broods of each size in groups according to the number of each sex present. For example, broods of three present four possible combinations: 3 ♂, 2 ♂ + 1 ♀, 1 ♂ + 2 ♀, 3 ♀. In table A are included only broods in which no deaths are known to have occurred. There are given the actual number of broods containing a given combination of males and females, likewise the "expected" number, to the nearest integer. In computing these last figures, it has been assumed that males and females are equally likely to be produced. The close approximation to equality in the material as a whole seems to warrant this procedure.

When we consider the comparatively small number of broods present in most of these groups, the agreement between the actual and the expected figures is remarkably close. This agreement is

TABLE A.

NUMBER OF BROODS CONTAINING EACH POSSIBLE COMBINATION OF MALES AND FEMALES.

Number in brood														
1	Combinations						1♂		1♀					
	Number of broods (actual)						64		61					
	Expected number						62		62					
2	Combinations					2♂		1♂, 1♀		2♀				
	Number of broods (actual)					56		120		66				
	Expected number					60		121		60				
3	Combinations				3♂		2♂, 1♀		1♂, 2♀		3♀			
	Number of broods (actual)				53		147		171		56			
	Expected number				53		160		160		53			
4	Combinations			4♂		3♂, 1♀		2♂, 2♀		1♂, 3♀		4♀		
	Number of broods (actual)			18		88		129		83		18		
	Expected number			21		84		126		84		21		
5	Combinations		5♂		4♂, 1♀		3♂, 2♀		2♂, 3♀		1♂, 4♀		5♀	
	Number of broods (actual)		4		22		27		29		17		5	
	Expected number		3		16		32		32		16		3	
6	Combinations	6♂		5♂, 1♀		4♂, 2♀		3♂, 3♀		2♂, 4♀		1♂, 5♀		6♀
	Number of broods (actual)	0		0		11		9		7		3		0
	Expected number	0		3		7		9		7		3		0

particularly striking when we compare the actual and expected totals for all of the broods in which all members were of the same sex. The actual number of such homosexual litters, among broods containing from 2 to 6 individuals inclusive, was 276. The most probable number on the assumption of purely random sex-production was 274.[1] Thus, not only do we find approximately equal numbers of males and females in the population as a whole, but in single broods the distribution follows the laws of chance. There is no tendency for fetuses (or germ cells) developing in the same parents at the same time to give rise to organisms of the same sex.

It may seem, on first thought, that such evidence is conclusive against the efficacy of any factor, except the chance meeting of the gametes, which might be supposed to play a part in determining the sex ratio. On the assumption, for example, that seasonal influences of an undetermined character may affect this ratio, should we not expect undue proportions of all-male broods at one time of year, and of all-female broods at another time, and should not this fact result in a preponderance of homosexual broods throughout the year as a whole? We have not computed the proportions of all-male and all-female broods by months, but the probability of the excessive production of such broods at certain times of the year may be granted. We must, however, point out the equal probability of an excessive production of evenly balanced broods at other times of the year. During these months of average sex ratios, we should presumably have not only equal numbers of males and females produced *in the aggregate,* but a tendency toward balanced broods on the part of *individual mothers.* The possibility of sex-determining agencies other than chance combinations of the gametes in fertilization is quite unaffected by these results.

One important conclusion seems justified, however, by this utter absence of any tendency toward the preponderant production of homesexual litters. This is the non-occurrence of polyembryony or true twinning, at least with sufficient frequency to affect the results.

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[1] If we include fractions (a more exact procedure) this number becomes 277. Surely this is a close "fit!"

Newcomb (1904), from a study of 7,896 families, concluded that, after making allowance for a slight preponderance of males, the sexes in each family were distributed according to chance. There was no tendency toward an excessive production of families consisting wholly of one sex. A consideration of multiple births, however, based upon French and German statistics, showed the existence of a pronounced tendency toward agreement in sex on the part of twins and triplets. Biologists would have confidently predicted such a situation, owing to the familiar phenomenon of duplicate ("identical") twinning, but Newcomb strangely overlooked this explanation of his results and found in them support for the idea that sex is determined by unknown causes operating during development.

RACE.

In the following table, the mice of "pure" (*i.e.*, non-hybrid) stock[1] are grouped according to the geographic race (subspecies) to which they belong. The La Jolla representatives of the subspecies *gambeli* are here kept distinct from those coming from the more northern localities (Berkeley and Calistoga). This is partly due to the appreciable morphological differences between these local races of *gambeli*, partly to the fact that the northern representatives were nearly all born during the months of May to August, a circumstance which may account in part for the extremely low sex ratio found in this group.

Subspecies.	Males.	Females.	Ratio.
gambeli (Berkeley and Calistoga)	49	72	68.06 ± 8.50
gambeli (La Jolla)	770	840	91.67 ± 3.07
sonoriensis	350	373	93.83 ± 4.70
rubidus	150	124	120.97 ± 9.91
Total	1,319	1,409	93.61 ± 2.36

In making a comparison between these races, we may reasonably leave out of consideration the small group of Berkeley and Calistoga *gambeli*. The two main groups (numerically speaking), *sonoriensis* and La Jolla *gambeli*, differ from one another by an amount which is less than half of its probable error. These two, however, particularly the latter, differ from *rubidus* by amounts which are possibly significant. But it is not certain that these figures imply the existence of actual racial differences in respect to sex ratio. One thing may be stated with confidence, however, namely, that the high ratio shown by *rubidus* is not due to any peculiarity in the seasonal distribution of its births. This has been shown by the following procedure. The sex ratio proper to each month, as computed for the pure races in general, has been weighted by the number of *rubidus* born in the corresponding month, and the product-sum divided by the total number of mice of this subspecies. The mean ratio thus obtained is 92.77, a figure even lower than the ratio for all mice of pure race.

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[1] Except *P. maniculatus dubius* and *P. eremicus*, for which the numbers are too small.

The existence of racial differences in the sex ratio have been pointed out for man. According to Newcomb (1904) and Heape (1909) the ratio is higher for the white race than for negroes. Heape (1907) likewise gives different figures for different races of dogs, these figures ranging from 96 to 136. There is little analogy, perhaps, between the artificial "races" of dogs and the geographic "races" of wild mammals, and the comparison with man is of equally doubtful validity.

HYBRIDIZATION.

Of the 4,652 young of known sex, 2,930 belonged to "pure" subspecies, 1,722 to subspecific hybrids. The former were not, of course, pure, in the sense of having been closely inbred for many generations, or of being uniform in genetic composition. Their purity was relative. Among the hybrids are included, not only those of the F_1, F_2 or later generations, but various back-crosses with the parent stocks as well.[1] This procedure seems warranted, according to any theory of the behavior of subspecific characters in hybridization. There is no clear segregation of the parental stocks in the F_2 generation, as would be expected if these stocks differed by only one or by a few pairs of unit factors. In consequence the F_2 and back-cross individuals are in a true sense *hybrids.*

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[1] Only subspecific crosses are here included. Crosses between "mutant" races within a subspecies, or between one of these and the parent stock, have not been regarded as hybrids for the purposes of this study. Of these, however, the number is not sufficient to materially affect the ratio for the "pure" stock, even though such an effect were known to occur.

The proportions of the sexes in the two lots are as follows:

	Males.	Females.	Ratio.
Pure	1,414	1,516	93.27 ± 2.32
Hybrids	881	841	104.76 ± 3.41

The difference between these two ratios is 11.49 ± 4.1, thus being slightly less than three times its probable error.[1] These figures by themselves, therefore, cannot be held to prove at all conclusively the existence of a higher sex ratio among subspecific hybrids in *Peromyscus.* But the probability of such a difference is greatly increased by several circumstances. (1) It has already been shown (Fig. 4) that the "hybrid" ratio exceeds the "pure" in nine months out of twelve, and that in two of the three exceptional cases the differences are trivial. (2) The hybrid ratio was higher in six of the seven years, taken separately (Fig. 7), and the single exception here is based upon a very small number of individuals. (3) The ratios for all of the five separate groups of hybrids are larger than that for the pure stock. (4) Such a difference accords well with the bulk of the evidence for other species of animals.

It is quite unlikely that the difference here found is due to any of the other factors with which the sex ratio of *Peromyscus* has been found to be correlated. As regards season, it has already been pointed out that this difference between the hybrid and pure material holds for nearly all the months of the year. Likewise the difference is very nearly the same when we deal, in each case, with the mean of the monthly sex ratios.

Again, as regards the size of the brood, the mean difference, in this respect, between the hybrid and pure stocks (3.26 and 3.20) is such that it could not exercise an appreciable influence upon the sex ratio.

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[1] It may be pointed out that when this probable error is computed according to a method widely used (see p. 127), the difference is more than five times the latter.

Nor can the differences be due to the varying proportions of these two groups which were born in different years. As already mentioned, the difference holds for six of the seven complete years of our records, the exception being unimportant, owing to the small numbers concerned. Likewise a computation analogous to that described on p. 139 shows that distribution by years (on the assumption of an equal sex ratio in hybrid and pure stock) could account for only a small fraction (2.5) of difference actually found.

Owing to the relatively small numbers, it is hardly worth while to discuss at any length the separate subspecific crosses which were made. The figures for the five different groups, with the numbers (in parenthesis) on which they are based are as follows: 114.63 (176), 114.11 (698), 97.27 (361), 95.65 (270) and 93.75 (217). Thus, all of these figures are higher than the ratio for the "pure" stock, though in the last case the difference is trivial, and in only one case is it as much as three times its probable error. Of the smallest of these figures it should be stated, however, that it is based entirely upon mice which were born from May to December, inclusive, thus missing the spring maximum.

All in all, the evidence, if not wholly decisive, points rather strongly to the conclusion that in *Peromyscus* hybridization *per se* results in increasing the proportion of males which are born.

Various previous writers have called attention to the larger proportions of males resulting from hybrid matings. To mention but a few of these cases, Guyer (1909) has presented evidence of this sort for various bird crosses,[1] Riddle (1917) for pigeons, Harrison (1919) for lepidoptera, Pearl and Pearl (1908) and Little (1919) for man, King (1911) for rats and mice. In certain of the cases discussed by Guyer and Harrison[2] the proportion of males reached 100 per cent.

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[1] In some of the bird crosses discussed by Phillips (1921) the males do not seem to be in excess, but the number of individuals is small, and the data are not presented with a view to answering this question.

[2] Harrison's results are complicated by the different behavior of various reciprocal crosses in respect to the sex ratio, by the preponderance of females in some cases, and by the appearance of "intersexes," *i.e.*, intermediates between the two sexes.

Miss King gives the data for 425 hybrids (F_1, F_2 and F_3) between the Norway and the albino rat. The sex ratio found was 119.07 or 118.11, depending upon whether the entire number was included, or whether consideration was restricted to the most reliable series (277). In either case, the difference cannot be regarded as significant, according to the standard here adopted. In the first case, it is 14.5 ± 8.3.[1] Miss King has also computed the sex ratio, from the records given by von Guaita of hybrids between albino mice and Japanese waltzing mice. The ratio is 113.17, but this is based on only 356 mice of known sex, and is surely of doubtful value.

Pearl and Pearl give the results of computations, based upon vital statistics of the city of Buenos Aires. They have compared the sex ratios resulting from Argentine × Italian and Argentine × Spanish matings with those resulting from matings within each "pure" race. The figures for the "hybrid" group were in each case the larger, and the authors believed that the difference was significant in three of the four cases taken singly. But when these probable errors are computed according to the formula now adopted by Dr. Pearl (see p. 127), the significance of these various differences becomes doubtful in all cases except one. That between the sex ratio for the Italian-Argentine cross and for pure Italian stock, is 4.95 ± 1.05. In all of the other cases the probable error is half or more times as great as the difference. Despite the great numbers comprised in these statistics, there is thus some doubt as to the reality of these differences. And their biological meaning is further obscured by the fact that the "pure" races differ among themselves in respect to their sex ratios quite as significantly as they differ from the "hybrids";[2] likewise by the fact that the highest "pure" ratio (the Spanish × Spanish) is very nearly identical with the lower of the two "hybrid" ratios. It should be added that the authors themselves did not express any great confidence that the higher ratios of the mixed matings are due to hybridization *per se*. We shall discuss later the significant yearly differences which are shown in Table I. of the paper of Pearl and Pearl, though not referred to by these authors.

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[1] Assuming 104.6 ± 2.77 as the normal ratio for the white rat.

[2] The same reservation must, though certainly with less cogency, be made in the case of our own data, discussed above.

Little (1919) has reported upon a smaller number of offspring of "pure" and "hybrid" stocks, born at the Sloane Maternity Hospital in New York City. The matings considered were those within or between the following "races": English, Irish, Scotch, Italian, Russian, Greek, Austrian and German. Of the births of "pure" stock, there were 5,753, and these gave a sex ratio of 106.27 ± 1.81. Of the "hybrid" births there were 1,305, giving a sex ratio of 121.56 ± 4.49. The difference between these ratios is a trifle over three times its probable error. In each case stillbirths were included. It is of interest to note that the proportion of stillbirths among the "hybrid" matings was considerably lower than among the "pure."

If the foregoing figures represent an actual biological difference between the two groups under consideration, the magnitude of this difference is surprising. For each of the "pure" races under consideration is, of course, not a race at all, biologically speaking, but is itself a hybrid mixture. The basis employed in these records was obviouslly nationality, not ethnic stock.

In a later paper (1920) Little has divided his material into "European pure," "European hybrid," "United States white," "British West Indian colored," and "United States colored." In general, the significance of Little's findings is greatly diminished if the probable errors are computed according to the formula which has been employed in the present paper. Thus computed, these are about double the errors given by Little. But at least three of this author's differences remain of probable significance, viz.: those between European pure (104.54) and European hybrid (122.86), between European pure (104.54) and U. S. white (118.33), and between U. S. white (118.33) and U. S. colored (96.12). It is of considerable interest that there is no significant difference between European hybrid and United States white. "This shows," as Little remarks, "that in the data studied, the United States white ratio is essentially that of a hybrid race," *i.e.*, an even more hybrid one than the European "races."

INBREEDING.

A comparison of "inbred" and "outbred" stock (as defined below) was made at a preliminary stage of these studies, when the total number of individuals dealt with was about 1,700 less than at present. The results of this comparison were so completely negative and the amount of work involved is so great,[1] that it has not been thought worth while to revise them.

For the purpose of comparing the sex ratios, our material was divided into two classes, "outbred" and "inbred." The first class included offspring from matings between unrelated individuals or individuals not related more closely than first cousins; the second included matings between parents and offspring or brothers and sisters.

In the first class, we have 2,346 individuals, comprising 1,171 males and 1,175 females. The sex ratio is 99.66 ± 2.70. In the second class, we have 1,087 individuals, comprising 547 males and 540 females. The sex ratio here is 101.30 ± 4.07. It is obvious that this difference must be regarded as accidental. When we restrict the "inbred" group to those individuals which were derived from the matings of full brothers and sisters, the sex ratio becomes somewhat larger, being 103.56 ± 4.82. But even here, the difference between this figure and that for the "outbred" group is much less than its probable error.

Various investigators have offered data which they believed to indicate an effect of close inbreeding upon the sex ratio. It is of interest (and quite characteristic of the literature of "sex determination" in general) that some writers have found a higher proportion of males in inbred strains, while others have found the females to be in excess.

Miss King (1918), from extended experiments on the white rat concludes that inbreeding *per se* does not affect the sex ratio. Her experiments yielded, however, one important positive result, namely that strains could be selected, giving preponderant numbers of male and female offspring respectively.

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[1] It is necessary, with every brood, not only to look up the birth records but to trace out the pedigrees to some extent.

ORDER OF BIRTH.

Both the order of birth, and the age of the parents have been regarded by various writers as factors in determining sex. From our data it would be difficult to separate the influence of these two factors, had we reason to suspect that either of them had any effect upon the sex ratio. Since, however, our findings here are quite inconclusive, it is a matter of no importance that the possible influence of the two factors should be kept distinct.

In 196 cases, it happened that the same parents had two or more litters of young. The sex ratio for the 554 individuals of known sex comprised in the first broods[1] is found to be 91.70 ± 5.26. That for the 627 mice in the second broods is 104.91 ± 5.65. This would seem to be a considerable difference, but the numbers are small and the probable errors correspondingly high. We are not, therefore, warranted in attaching any significance to this difference. When we add the very limited number of third and fourth broods to the second ones and compute the sex ratio for the 738 mice of known sex belonging to these "later" broods, we have 103.31 ± 5.48. The probability of an actual difference in sex ratio between first and later broods is actually diminished by this procedure.

It is of some interest that the second broods averaged somewhat larger than the first, the mean figures being 3.39 and 3.05 respectively, based upon 196 broods of each class. This difference is nearly three times its probable error.

King and Stotsenburg report for the white rat a steadily decreasing sex ratio in passing from the first to the fourth litters borne by the same mothers, the first figure being 122.0, the last 74.5. The authors recognize, however, that the number of broods under consideration does not warrant any final conclusions on this point.

Punnett (1903) from an examination of Burke's "Peerage" concludes that the first born, in man, are predominantly males.

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[1] By "first" broods we here mean the first which are known to have been borne by a given mother. Since, in more than a third of the cases, the mothers were wild mice, which had been trapped when nearly or quite mature, it is likely that many of these had already given birth to young. In such cases, the actual contrast is between *earlier* and *later* broods.

Newcomb (1904) likewise finds that "the first born child of any mother is more likely to be a male, in the proportion of about 8 to 7" (p. 28).

NUTRITION.

Our data on the much-discussed subject of the relation of nutrition to the sex ratio are extremely meager, though it seems worth while to include them in this report. Any such extreme effect as has been alleged by certain writers might have been expected to manifest itself, even in the limited numbers here considered.

For the purpose of testing the possible beneficial effect of including meat in the dietary of the stock, certain lots of mice, in three successive generations, were divided into two sections, one being given a rather liberal supply of chopped, boiled meat (commonly liver), in addition to the regular ration which was strictly vegetarian, the other (control) lot being restricted to the latter. The experiment was commenced at about the time when the first of the three generations referred to was mated. The meat diet was continued until the mice of the third generation were fully grown.

In all, there were 237 young of determined sex among the offspring of meat-fed parents (second and third generations). These gave a sex ratio of 104.31 ± 9.13. In the control lot were 228 young, giving a ratio of 96.56 ± 8.61. Here, again, it is obvious that the difference is not significant. The transfer of a very few individuals from the male to the female column (or vice versa) would be sufficient to reverse it.

Cuenot and Schultze (both cited by Morgan, 1907, pp. 385, 386) performed experiments upon rats and mice, respectively, with a view to testing the possible effects of feeding upon sex determination. In neither case were significant differences found between the sets of animals under comparison, but the numbers were very small and the results almost valueless statistically. More recently, Slonaker and Card (1918) have computed sex ratios for omnivorous and strictly vegetarian white rats, finding these to be .113.6 and 107.6 respectively. Since the number of individuals and other essential matters are not stated in the brief preliminary communication referred to, we must suspend judgment on the significance of these figures. Most recent experiments with other animals have given negative results as regards the effect of diet upon the sex ratio.

THE YEAR.

We have already referred to the surprising fact that the sex ratios of mice born in different years may differ widely and significantly from one another. The following table gives the figures for all the years comprised in our records, with the exception of 1914, for which the data are very meager:

	Males.	Females.	Ratio.
1915	307	306	100.33 ± 5.44
1916	262	209	125.36 ± 7.82
1917	175	248	70.56 ± 4.70
1918	758	736	102.99 ± 3.67
1919	137	116	118.10 ± 10.03
1920	121	138	87.68 ± 7.37
1921	496	560	88.57 ± 3.68

As judged by the customary standard, some of these differences are highly significant. That, for example, between 1916 and 1917 is 54.80 ± 9.1, the difference being six times its probable error. The chances that this result was due to "accident" (*i.e.*, that no difference would have been found if our numbers had been indefinitely great) are only about 23 in a million. The difference between the ratio for 1916 and the ratio for the entire period (97.37 ± 1.93) is 3.5 times its P. E., that between 1917 and the general ratio being 5.3 times its P. E. The difference between the largest figure (125.36) and the second smallest (87.68) is 3.5 times its P. E., that between the smallest figure (70.56) and the second largest figure (118.10) is 4.3 times its P. E. The difference between the second largest and the second smallest ratios is, however, only 2.5 times its P. E. Finally the difference between the ratios for the two years during which the greatest number of births occurred (1918 and 1921) is 14.42 ± 5.2, *i.e.*, it is 2.8 times its P. E.

The foregoing probabilities are, to be sure, not wholly cumulative, but the conclusion seems hardly avoidable that certain of them are real in the sense of not being due to errors or random sampling. We must, of course, consider the possibility that these differences among the sex ratios of the various years are due to some of the other factors already considered. May they not, in part at least, be due to the unequal representation in these different years of broods born in months of high or of low sex ratio? Aside from the fact that these yearly differences are even more extreme than any of the seasonal ones, we have excluded this possibility by a procedure previously adopted in similar cases. The number born in each month of each year was multiplied by the sex ratio for that month (based on the entire material). The weighted mean for the year in question was then computed. None of the yearly ratios thus obtained differed by more than 4.0 from the general ratio (97.37), showing that seasonal distribution cannot account for these yearly differences.

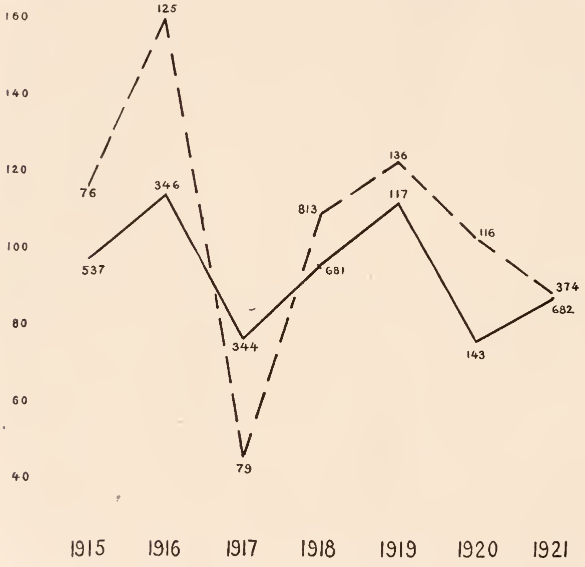

FIG. 7. Differences in the sex ratio during the different years comprised in our records. Continuous line = pure. Dotted line = hybrids.

Nor are the latter to be accounted for by the possibly unequal distribution of pure and hybrid births. Figure 7 shows that the same year-to-year fluctuations were undergone by both pure and hybrid stock, with the exception of the last (from 1920 to 1921).

When each of these years is divided into 3-month periods (February to April, etc.), we find that, on the whole, the years of low and high ratio show this tendency for each of the four "seasons." Graphs (not here reproduced) show that the "curve" for 1917, while undergoing closely similar fluctuations to that for the entire series, remains throughout far below the latter. Of the other two "low" years, 1921 remains throughout below the mean, while 1920 gives a lower ratio in three out of four of these periods. Of the two years which show ratios appreciably higher than the mean, each gives a "curve" which keeps far above the mean during three of the four seasons. The exceptions fall at different times in the two cases.

These considerations, while they add little to the evidence for the statistical reality of these yearly differences, show that the latter do not depend upon conditions which act primarily during any particular period of the year. To be sure, computations have been made which show that the departures of these yearly ratios from the mean condition occur preponderantly in the fall and winter months. This relation is probably due to chance, however, so that the figures need not be given here.

Similar differences have been recorded by a number of investigators between the sex ratios of different years for mankind, but so far as we know, these differences have not been discussed by them. They have been regarded as accidental, or at least as irrelevant and negligible. Thus in the study by the Pearls, already referred to, of the sex ratios of "pure" and "hybrid" races in the city of Buenos Aires, the authors confine their discussion to the apparently higher ratios which are found among offspring of mixed parentage. They quite overlook the fact, however, that the sex ratio of the entire population varies materially from one year to another, and that these yearly differences are in some cases both absolutely greater and statistically more certain than are those between the pure and hybrid elements of the population. Thus the ratio for the year 1900 is 97.0 ± .90, while that for 1904 is 105.4 ± .95. Each of these figures is based upon over 20,000 births, and the difference is 6 times its probable error. Even greater and more "significant" differences are to be found in the yearly ratios of some of the separate population groups (in one case 8 × P. E.), and it is of interest here to note that the years of high and low sex ratios do not tend to correspond in these various groups, but that they sometimes show exactly opposite conditions.

It is, of course, inconceivable that a difference of calendar year, as such, should have any more influence upon the sex ratios of mice or men than the phases of the moon or the conjunctions of the planets. It is likewise improbable that any mean difference in the weather from one year to the next, can be the responsible factor. For the meteorological differences between the most widely unlike years are small in comparison with the differences between the summer and winter seasons in any single year. But we have seen that these yearly variations of sex ratio may be even greater than the seasonal ones. Again, examination of the composition of the stock during these widely divergent years (say 1916 and 1917) gives no suggestion of a clue based upon considerations of this sort.

Huxley (1920) discusses a case in which a teleost fish produced, for nearly a year, three times as many females as males. Later, this ratio among the young produced changed to 2 females: 3 males for a few weeks, after which the numbers of the sexes became approximately equal and remained so for several years. Huxley believes it probable that in the first stage a certain proportion of the individuals having the zygotic formula XY became "somatic females" or "feminized males," and that such individuals produced X and Y eggs, which by fertilization gave rise in the next generation to an excess of males. A converse hypothesis might, of course, be invoked to explain the change in our stock, from a ratio of 125 in 1916 to one of 71 in 1917. But this is suggested merely as a remote possibility, justified only by the utter absence of any plausible scientific explanation to cover the case.

Finally, it should be urged that even highly improbable things sometimes happen, and that it is not impossible that our most "significant" figures may result from chance. An illustration of this fact, drawn from the experience of the senior author, is worth recording here. Two series of mice had been subjected to a difference of treatment which even the most ardent believer in "sex determination" by physical agencies would not think of as being effective in this connection. The difference was that in one series the right sciatic nerve was cut, in the other the left. The first 9 broods of "right" parentage consisted of 14 females and no males. During the same period, 5 broods were born of "left" parentage, consisting of 10 males and 4 females. Had the attempt been made to influence sex by experimental procedure, the result would have seemed highly satisfactory. Thus, the chance of obtaining 14 individuals which were exclusively females is only one in 16,384, and the "significance" of the results is increased when we consider the high preponderance of males in the contrasted group. Experiences such as this lead one to demand higher statistical probabilities than are frequently accepted as convincing.

SUMMARY.

Data have been presented, based upon over 4,600 deer-mice of known sex, which were born and reared in captivity, under temperature conditions not far different from those existing in nature. The following results seem to be of most importance.

1. In size the broods ranged from 1 to 9, the mean of the 1,567 broods being 3.22.

2. The sex ratio for the entire lot was 97.37 ± 1.93. When we include only those broods in which no deaths are known to have occurred (nearly nine tenths of the whole) the figure becomes 98.01 ± 2.07. Broods known to have been incomplete give a ratio of 93.08 ± 5.25. In order to eliminate the effect of seasonal differences, the mean of the monthly means has been computed. This is 95.65. None of these figures can be regarded as differing significantly.

3. The probable errors employed throughout this paper are based upon a formula different from that which has been used by various previous writers. As a result, the errors here given are about twice as great as would formerly have been computed, and

the probabilities claimed by us are correspondingly lower. According to this safer criterion, some of the most interesting of our differences are not decisively "significant."

4. Considering our aggregate material, there seems to be a definite seasonal cycle in the proportion of males and females born. The sex ratio presents two annual maxima, in March–April and August–October, respectively; and two annual minima, in winter and summer, respectively. The lowest figure is 78.79 (± 6.93), in November and the highest 113.04 (± 8.04) in April. This difference is about 3¼ times its probable error, and would ordinarily be regarded as "significant." A well-marked biennial rhythm is shown by both pure and hybrid stock, taken separately, though the fall maximum occurs in different months in the two cases. On the other hand, when our material is subdivided in certain other ways, the results are in some cases highly contradictory. We cannot, therefore, regard the existence of a seasonal cycle in the sex ratio of *Peromyscus* as being proved conclusively by our data.

In any case, the position of these annual maxima and minima, as found by us, does not correspond with those which have been reported for the white rat or for man. Indeed, the conditions in *Peromyscus* are very nearly the reverse of those described by certain other authors.

5. Subspecific hybrids (1,722) give a mean ratio of 104.76 ± 3.41. Mice of "pure" race (2,930) give a mean ratio of 93.27 ± 2.32. Considering these figures alone, the difference is barely significant statistically, but its reality is borne out by various other considerations. Furthermore, such an effect of hybridization upon the sex ratio was to be expected in view of the findings of various other biologists, working upon widely different organisms. It is possible, also, that the several subspecies of *Peromyscus* differ *inter se* to some extent.

6. A positive correlation exists in our material between the size of the brood and the sex ratio. Broods containing one to three individuals give a mean sex ratio of 94.85 ± 2.94, ones containing four to nine individuals give a mean sex ratio of 102.42 ± 3.01. The magnitude of these probable errors raises the suspicion, however, that these differences are accidental, and

our doubts are further increased by the lack of a uniform gradation when the broods are grouped according to size.

7. When the number of each possible combination of males and females, in broods of each size, is compared with the number expected according to chance, the conformity is found to be, on the whole, very close. For example, the number of all-male and all-female broods (excluding broods of one) was 276, the "expected" number being 274. There is thus no preponderant tendency toward the production of homosexual litters, and thus no likelihood that polyembryony or true twinning is at all common in these animals.

8. In our material, the sex ratio is lower for the earlier broods (91.7) than for later broods of the same mother (103.3). The numbers are so small, however, that the difference is probably accidental.

9. Likewise, inbreeding and outbreeding seem to have had no influence upon the relative numbers of males and females, within the limited material available for this comparison.

10. Similarly negative results were obtained from a comparison of the offspring of meat-fed individuals with the offspring of those whose diet was strictly vegetarian. Here again, the numbers were too limited to permit us to regard this experiment as decisive.

11. The most significant result of all, statistically speaking, and one which is at present utterly inexplicable, is the fact that the sex ratios for the seven different years included in our records show a wide range of variation. The extreme figures are those for 1916 and 1917, the ratios being 125.36 ± 7.82 and 70.56 ± 4.70, respectively. These figures are based upon 471 and 423 individuals, respectively. The difference is 54.80 ± 9.1 (*i.e.,* difference $= 6 \times$ P. E.). The likelihood of obtaining such a result by "accident" is less than one in 40,000. We have furthermore determined that this difference is not due either to the seasonal distribution of births, to the preponderance of hybrid births in one year, as compared with another, or to the operation of any of the other factors previously considered.

Mention was likewise made of the occurrence of similar annual differences in the sex ratio of man as revealed by the tables of

Pearl and Pearl (1908). Some of these differences we have shown to be much more highly "significant"—according to accepted standards—than the differences between the pure and hybrid races with which these authors were concerned.

Discussion.

In a field as well tilled as that of sex determination, it is both impracticable and undesirable for us to enter into any extensive review of the literature, and we offer no apology for the inadequate references to other workers which we have found it possible to include in the foregoing pages. Those who are unfamiliar with the literature of this field are referred to works by Geddes and Thomson (1914), Marshall (1910), Morgan (1913), and Doncaster (1914). The last two of these books are of particular value in this connection and supplement one another very nicely, since the viewpoint of the authors differs rather widely.

In recent years the view has been gaining ground that the sex of the individual is determined by the chromatin constitution of one or the other of the germ cells. In the majority of cases which have been investigated, it is believed that the differential factor is contained in the sperm cell, though there is evidence in a number of instances that the ovum is the gamete concerned. The cytological and genetic data upon which these conclusions are based are too well known to be discussed here. For many organisms the evidence is doubtless wholly convincing.

Many well-attested cases exist, however, in which the sex ratio is known to be correlated with certain external factors. Those who wish to universalize the chromosomal theory of sex determination, dispose of such cases by assuming the existence of either a differential mortality among the developing organisms, or a differential mortality among the sex-determining gametes, or some other factor favoring one class of gametes or zygotes, as compared with the other. It is assumed by such biologists that the sex of the organism is invariably predetermined in the nucleus of the fertilized egg, and is not subject to reversal by any influence that can be brought to bear subsequently.[1]

[1] Morgan (1913), for example, devotes a section of a chapter to "The Abandoned View that External Conditions Determine Sex," though there are reasons for believing that Professor Morgan does not take such an extreme position at the present time.

Other biologists, while granting this absolute predetermination of sex for some organisms, believe that in other cases the condition of the nucleus may be neutral in this regard, and that which of two potentialities shall finally prevail may be determined by chemical or physical influences acting upon the developing organism. There is, indeed, evidence which seems irreconcilable with the view that sex is always predetermined in the nucleus of the fertilized egg. We think, accordingly, that this second position is the safer one in the present state of our knowledge.[2]

As regards the data here reported for *Peromyscus,* we believe that (granting the reality of certain of the differences found) they lend themselves perhaps equally well to either of the above conflicting viewpoints. There may, for all we know, be seasonal differences in the relative production of male-producing and female-producing spermatozoa. Or, one kind may be more active at one time of the year than another. Or, the ova may so vary in their chemical composition that their attractive influence upon one or another type of spermatozoön varies with the season. Any or all of these things may be true, though there is not a scintilla of evidence that such is the case.

As regards the effect of crossing upon the sex ratio, this too, is reconcilable with either viewpoint. There might, in one way or another, be a preponderant selection of male-producing spermatozoa in hybrid fertilizations. On the other hand, the increased vigor which frequently accompanies hybridization might well influence the metabolism of the parents, and thus affect that of the developing fetuses.

When we come to those fluctuations in the sex ratio which are met with from one year to another, we must admit that their causes are utterly obscure. In the case of *Peromyscus,* it is difficult to conceive of any scientific explanation whatever for the fluctuations found. Even when we admit the likelihood that environmental conditions may play a direct or indirect part in sex determination, it is hard to see how the relatively slight differences which distinguish one year from another could bring about a greater effect than the vastly larger differences which distinguish the seasons of a single year. The calendar year, as such, would seem to be as devoid of causal efficacy in this matter as are the planetary influences which are invoked by the astrologists.

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[2] This is the viewpoint adopted by Doncaster (1914, Chapter X.).

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BIOLOGICAL BULLETIN

SELECTIVE FERTILIZATION AND THE RATE OF POLLEN-TUBE GROWTH.

D. F. JONES,

Conn. Agric. Exper. Station, New Haven, Conn.

A difference exists in the ability of pollen from dissimilar plants to accomplish sexual fusion when acting in competition. This has been shown by the writer (1920) with maize (*Zea mays* Linn.) and the tomato (*Lycopersicum esculentum* Mill.). Two other cases of a similar effect have been reported. These will be referred to later. In these species the plant's own pollen is more efficient in completing fertilization than pollen from plants having somewhat different genetical construction, and with maize the superiority of self-fertilization is greater as the germinal differences increase.

The method used to prove that there was an inequality in fertilization with maize was to make a mixture of approximately equal quantities of pollen from two different lots of plants, each of which possessed a dominant seed character. The mixture was applied at the same time to the plants which furnished the pollen, and when the seeds were mature it was possible to separate the seeds resulting from the two kinds of pollen on both types of plants. For example in some of the tests a white, smooth-seeded variety was contrasted with a yellow wrinkled variety. In one case the self-fertilized seeds were white and the cross-fertilized yellow, and in the other the contrast was between wrinkled and smooth seeds. The four classes of seeds in the two types of inflorescence were quite distinct and easily separated and counted. The numbers when arranged in the form of a proportion showed that there was a marked selective action in favor of self-fertilization.

The greatest difference in fertilizing efficiency was found when a small seeded variety of popcorn of the *Zea mays everta* type having very corneous and pointed seeds was used. The selective action was so pronounced that it seemed worth while to repeat the tests with this type on a more extensive scale. At the same time, the pollinations were made and the data recorded in such a way as to give an indication as to whether or not the result was due to a differential rate of pollen tube growth.

The seeds of maize are arranged regularly on a central spike. Each ovule has a separate pistil and these form a mass of fine filaments which extend beyond the enclosing leaf sheaths. See Fig. 1. Just before the pollinations were made the pistils were cut off evenly at a short distance beyond the tip of the spike, and the pollen mixture was applied to the cut ends of the filaments. The distance that the pollen tubes had to travel to reach the ovules differed considerably in the case of the seeds produced in the tip of the spike as compared with those at the base. The mature pistillate inflorescences of the material worked with ranged from ten to twenty centimeters in length. At the time fertilization took place they were considerably shorter than this. It is estimated that the pollen tubes travelled through a distance which varied from about five to fifteen centimeters. If the plant's own pollen tubes grow faster than the foreign tubes, we would expect fewer cross-fertilized seeds at the base of the spike than at the tip.

Five different mixtures of pollen were made and applied to about ten plants of each of the two contrasted types. For the white, smooth-seeded type a first generation hybrid of two inbred strains of a variety of sharply pointed popcorn known as Squirrel Tooth was used, and for the yellow, wrinkled type another first generation hybrid of two inbred strains from a variety of sweet-corn known as Golden Bantam. Hybrid plants were employed because of their size and vigor, making it possible to secure a large number of seeds from a single application of pollen. The plants of each type were characteristically uniform, and were practically identical in genetical constitution. They were producing segregating gametes but these were presumably alike in respect to their cytoplasmic covering. In any case the gametic differences were no greater than are present in the original varieties. The same two types of plants were used in all five pollen mixtures. The mature inflorescences resulting from the application of mixed pollen were divided into two approximately equal halves and the

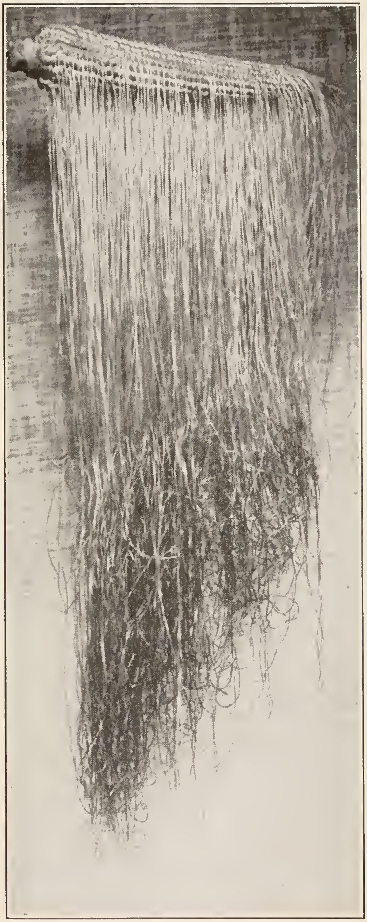

FIG. 1. The pistillate inflorescence of maize at the time of pollination with leaf sheaths removed. (About one half natural size.)

seeds from the top half and bottom half were counted separately. The data from the entire spikes are brought together and given in Table I. to present the amount of selective action shown. The figures from all the plants used with each mixture are combined and are arranged in four classes in the form of a proportion. If there were no differences in fertilizing ability the proportion should be a perfect one within the limits of random sampling. The white, smooth-seeded plants are designated *A* in the tables, and the plants with yellow wrinkled seeds when uncrossed are listed under the heading of *B*. The number of seeds resulting from *A* pollen and from *B* pollen on *A* plants should be in the same ratio as the numbers from the same two kinds of pollen on *B* plants irrespective of the amount or viability of each kind of pollen in the mixture. As in the numerous cases previously reported such is clearly not the result obtained, as there are much fewer cross-fertilized seeds than would be expected if fertilization took place at random. The selective action is in favor of the homogeneous union as was found before and the differences are marked. The last column gives the deviation from the closest calculated perfect proportion based upon the per cent. of cross-fertilized and self-fertilized seeds on each type of plant. The maximum deviation possible is 50 which would indicate complete functioning of each kind of pollen on its own flowers, but not at all on the others, or vice versa as the case might be. The deviations actually range from 12 to 41 in favor of self-fertilization, in each case, and show a very high degree of selective action, amounting in some cases to almost total inability of the extrane-

TABLE I.

THE AMOUNT OF SELECTIVE ACTION SHOWN BY MAIZE IN FIVE POLLEN MIXTURES.

Pollen Mixture No.	Number of Seeds.				Total Number of Seeds.	Deviation from Perfect Proportion in Per Cent.
	A × A.	A × B.	B × A.	B × B.		
1	811	11	381	2,006	3,209	41.35
2	4,222	27	466	1,404	6,119	37.22
3	1,568	2	319	224	2,113	20.56
4	1,930	29	73	309	2,341	39.71
5	4,084	6	963	290	5,343	11.50

ous pollen to bring about fertilization when acting in competition. Fig. 2 shows a pair of mature pistillate inflorescences resulting

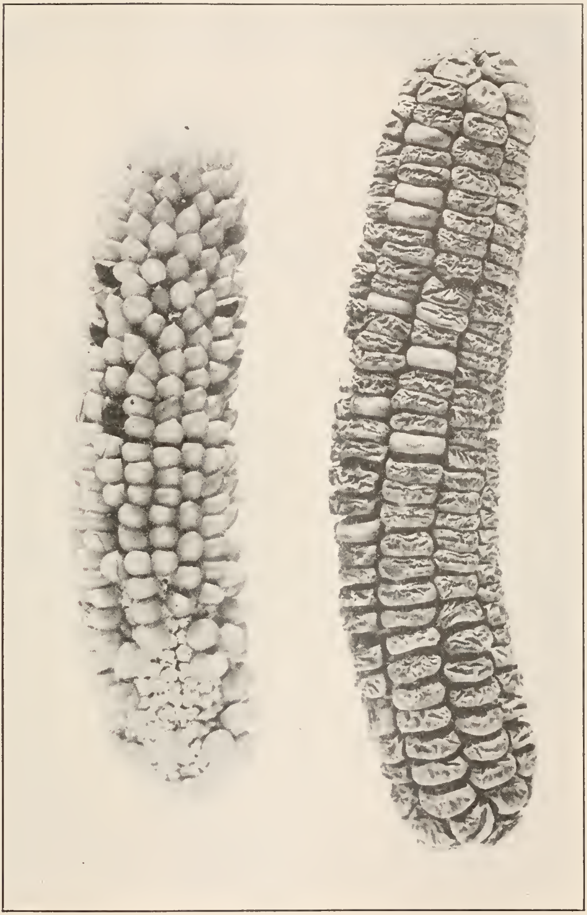

Fig. 2. Mature inflorescences resulting from the application of a mixture of pollen from the two kinds of plants which produced them. (About three fourths natural size taken on orthochromatic plate with Wratten C blue color filter.)

from pollen mixture number 1. The cross-fertilized seeds stand out unmistakably on both plants, but are few in number.

The data which show that this selective action is due in part, at least, to differences in rate of pollen-tube growth, are arranged in Table II. The total number of seeds in the top and bottom halves of the inflorescences, as divided arbitrarily before shelling, are roughly equal. The number of cross-fertilized seeds per hundred of all seeds is more in those seeds which resulted from the shorter lengths of pollen tubes. Only one exception is noted, and here there were only six crossed seeds in a total of over four thousand. In all the others, positive differences are shown, but these, however, are not large, so that one cannot be sure whether or not the inequality in fertilization is due entirely to differences in the rate of pollen-tube growth. Corroborative evidence has been furnished by Miller (1919), who has observed that many pollen tubes may start to grow down the style of maize, but in about 100 examinations only one tube was seen to reach the ovary cavity in every case.

TABLE II.

THE DISTRIBUTION OF CROSSED SEEDS IN MAIZE INFLORESCENCES RESULTING FROM MIXED POLLINATIONS.

Pollen Mixture No.	Plant Type.	Number of Seeds.		Total Number of Seeds.	No. of Crossed Seeds per Hundred.		Excess of Crossed Seeds per Hundred in Top Half.
		Top Half.	Bottom Half.		Top Half.	Bottom Half.	
1.......	*A*	452	370	822	1.99	.54	1.45
1.......	*B*	1,219	1,168	2,387	17.23	14.64	2.59
2.......	*A*	2,126	2,123	4,249	1.22	.05	1.17
2.......	*B*	979	891	1,870	26.66	23.01	3.65
3.......	*A*	811	759	1,570	.25	.00	.25
3.......	*B*	298	245	543	60.07	57.14	2.93
4.......	*A*	1,023	936	1,959	2.54	.32	2.22
4.......	*B*	186	196	382	24.73	13.78	10.95
5.......	*A*	2,075	2,015	4,090	.10	.20	−.10
5.......	*B*	612	641	1,253	78.43	75.35	3.08

A similar selective action favoring the plant's own pollen has been found with cotton. Balls (1919) put an equal quantity of pollen of two distinct cultivated types of this plant (*Gossypium*) on the stigmas of each. Only ten hybrids were obtained from 330 seeds resulting from the mixed pollen. Likewise Heribert-Nilsson (1920) found that the pollen tubes of *Œnothera gigas* grow slower in the styles of *O. Lamarckiana* than do the latter's own pollen tubes. These results were obtained by cutting off the styles close to the ovary at different times after pollination and noting the shortest time after pollination necessary for seeds to set.

A somewhat different kind of selective fertilization has been demonstrated by Correns (1920) with the diœcious plant *Melandrium.* The staminate plants of the species worked with are heterogamous, and the pistillate plants homogamous, corresponding to the sex conditions in animals of the *Drosophila* type. The pistillate-determining pollen tubes apparently grow faster than those which result in staminate plants. This was demonstrated in the following way. The mature seed-pods were divided into upper and lower halves and the seeds of each grown separately. The seeds from the upper portion resulting from the shorter lengths of pollen tubes gave 68 per cent. pistillate plants, while the seeds from the lower portion gave 56 per cent. of the same type. It should be noted that the conditions are reversed in the case of *Melandrium* and *Zea.* In the former the pollen tubes enter the ovary at a common point and are free to fertilize the first ovules they reach. The tubes which grow fastest therefore fertilize the ovules in the upper part of the ovary, leaving the slower-growing tubes to pass on down to the lower part. In *Zea* each ovule has a separate style so that, the longer the distance to traverse is, the less chance will the slower-growing tubes have of reaching the goal first.

Correns also found that when pollen was applied in large excess as compared to a moderate application, the pistillate plants resulting were always in greater proportion. But even when a deficient amount of pollen was applied there was still a small excess of pistillate plants showing that some other selective factor than differences in rate of pollen-tube growth was operating in addition.

Heribert-Nilsson also obtained aberrant ratios from self-fertilized and back-crossed heterozygous red-nerved plants of *Œno-*

thera Lamarckiana. He interprets the result as due to a selective action between gametes carrying different factors. In this case as well as in the preceding experiments with *Melandrium* two kinds of gametes were produced by the same individual and presumably they were alike in respect to their cytoplasmic covering, differing only within the nucleus. In the case of *Zea, Lycopersicum, Gossypium,* and *Œnothera Lamarckiana* pollinated by *gigas,* the gametes came from different individuals of unlike germinal construction and therefore differed both in nuclear content and enveloping cytoplasm. The two manifestations of selective fertilization may be unrelated. On the other hand it is not unlikely that they may have something in common.

The discrimination which works against the bringing together of individuals of unlike germplasm has been demonstrated by representatives of four distinct orders in the two main classes of angiosperms. In its effect it is comparable to the assortative mating of animals from the lowest to the highest. The predilection for the bringing together of like with like is more evident as the germinal differences increase. There is thus exhibited a tendency which when carried far enough may erect an impassable physiological barrier between different groups that were once compatible. It is an indication that sterility between species is the result of the accumulation of genetic differences, however these differences may arise.

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THE TEMPORARY CONCENTRATION OF SEA-SALTS ABOUT *ARBACIA* EGGS.

OTTO GLASER.[1]

I. Introduction.

If the precipitates prepared by Miss Woodward[2] and myself[3] have been correctly understood, Lillie's hypothetical fertilizin[4] is a mixture of at least two chemical entities. On this view, therefore, we cannot assume that the sperm-agglutination test, however reliable for agglutinin, is necessarily also a measure of the concentration of the associated lipolysin. The ratio $\frac{\text{agglutinin}}{\text{lipolysin}}$, may of course be a constant; yet we cannot know this until we find out.

With this problem in mind, I began, last summer, to search for other methods; the ideal being some convenient procedure which would eliminate the physiological variables and leave to the observer nothing except a reading. Experiments on specific gravity, surface tension and viscosity naturally suggested themselves. Changes in one or all of these properties of sea-water might be expected as exudate leaves the eggs and becomes distributed in the solvent. Such changes, plus or minus, should stand, within certain limits, in some direct or inverse relationship with the concentration of the organic constituents of the secretion. A comparison of such values with results gotten by the sperm-agglutination test for the same secretions and for solutions of precipitated agglutinin having the same specific gravity, surface tension, or viscosity, could then be used to throw light on the ratio $\frac{\text{agglutinin}}{\text{lipolysin}}$.

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[1] From the Biological Laboratory of Amherst College, Amherst, Mass.

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[4] Lillie, F. R., The Mechanism of Fertilization. Science, Vol. XXXVIII, p. 524.

II. The Diluent Effect of *Arbacia* Eggs.

I tried practically all the familiar variants of the drop and capillary methods for surface tension; the rates of flow, of falling plungers, and of capillary rise, for viscosity; the Westphal balance, for specific gravity. Yet despite the precautions taken to insure comparable measurements, my results remained inconsistent. Even the specific gravity readings were irregular and their sense totally contrary to expectations. In fact the specific gravity of sea-water in process of receiving exudate from the eggs, rarely rose, never remained constant, and almost invariably fell. The records in Table I. show the discrepancies.

Table I.

No.	Ratio.	Secretion Time.	Temperature.	Specific Gravity.	
				Sea-Water.	Exudate.
A.....	$\frac{\text{eggs}}{\text{sea-water}} = 1/10$	1 hr.	23°	1.0226	1.0222
B.....	" = 1/10	1 hr.	23°	1.0226	1.0217
C.....	" = 1/10	1 hr.	23°	1.0226	1.0222
D.....	" = 1/10	2 hrs.	21.5°	1.0227	1.0231
E..	" = 1/10	30 min.	23°	1.0231	1.0222
E..	" = 1/10	1 hr.	23°	1.0231	1.0221
E..	" = 1/10	1½ hrs.	23.5°	1.0229	1.02195
E..	" = 1/10	2½ hrs.	23.7°	1.02275	1.0224

It is apparent that the introduction of *Arbacia* eggs into sea-water results in a slight decrease of specific gravity and that this decrease may be compensated or even over-compensated with the lapse of time. This is illustrated by series *E*, based on a single set of eggs, and by *D*. If their correctness could be established, these observations would account for the inconsistencies in surface tension and viscosity determinations; yet the fall in specific gravity, the irregularities in the magnitude of the fall, and the compensations would remain to be accounted for.

III. The Chlorine Deficit in Egg-Secretion.

There are four possible explanations. First, the specific gravity determinations may have been wrong. Secondly, the diluent effect of the eggs might be due to a liberation of heat. And finally, the reductions in density may have resulted from one or the other or both of two causes; either salts are removed from the sea-water directly by the eggs, or the exudate itself affects the sea-water such that the specific gravity must fall.

The first two assumptions were entirely ruled out by subsequent developments. I shall limit myself therefore to a discussion of the other possibilities. Do *Arbacia* eggs abstract salts from the sea-water or is the reduction in specific gravity an effect traceable to the materials which the eggs secrete?

Just how the presence of exudate might reduce specific gravity, is more or less uncertain in detail. Nevertheless this possibility must be reckoned with, both in its thermal, as well as more narrowly chemical, aspects. Salts dissolved in water apparently bring about a "contraction" of the solvent.[5] Where ionization is incomplete, this effect, though marked, is not easily calculable; in dilute solutions, however, the total "contraction" is additively the sum of specific effects or moduli of the individual ions. Thus a gram-molecule of a salt with molecular weight M in m grams of water produces a change of volume Δ_v such that

$$\Delta_v \equiv \frac{M - m}{S} - \frac{m}{S_0},$$

where S is the density of the solution at a given temperature and S_0 the density of pure water at the same temperature.

Since undissociated molecules also have a "contractile" effect, and since each ion has a specific modulus, it would be quite possible to bring about a reduction in the specific gravity of sea-water by the addition of some agent that disturbs, selectively or otherwise, the ion-salt equilibrium. Our problem then narrows down to this: is the observed decrease in specific gravity associated with a genuine salt-deficit in the solution or is it the outcome, direct or indirect, of a physical-chemical rearrangement among the free solutes?

If real, and essentially non-selective, a salt-deficit in the solution should be detectable by the titration of the chlorides. For this purpose I used $n/20$ $AgNO_3$; and two or three drops of 10 per cent. K_2CrO_4, in doubly distilled water, as indicator.

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[5] Nernst, W., Theoretical Chemistry. MacMillan and Co., London, 1895, p. 331.

Special precautions were taken since the specific gravity readings suggested that differences, if at all discoverable by titration, would be small. For this reason the measurements throughout were made with the same burettes and pipettes and comparable titrations always at the same temperature. Moreover it soon became apparent that the preparation of the eggs could not be carried out by any of the methods in ordinary practise. I therefore washed the sea-urchins first very thoroughly in a stream of running fresh water after which they were completely submerged in dishes for from three to five minutes. The bath was followed by partial drying and the complete removal of the spines by means of a coarse cloth. The naked tests were then carefully wiped with a clean towel and placed in an inverted position in individual Syracuse watch crystals. This procedure may appear cumbersome. However, it consumes very little more time than the usual methods of preparation and is the only way in which eggs absolutely free from detritus, traces of sea-water, dermal and other secretions, can be gotten. Incidentally the method has a further advantage; the brief immersion in fresh water causes the sea-urchins to shed their sexual products in unusual quantity and with the greatest promptness. Indeed one must work quickly in

Table II.

Series.	Ratio.	Secretion Time, Minutes.	Chlorine, in c.c. $n/20$ AgNO$_3$, per c.c.		Deficit per c.c. in 1/10 Milligrams of Chlorine.
			Sea-water.	Secretion.	
I.....	$\frac{\text{eggs}}{\text{sea-water}} = 1/7$	15	10.5	10.3	−4
II.....	" = 1/7	15	10.5	10.4	−2
III.....	" = 1/30	60	11.0	10.9	−2
IV.....	" = 1/8	60	10.1	9.8	−5
IV.....	" = 1/8	60	10.0	9.8	−4
IV.....	" = 1/8	60	10.0	9.9	−2
IV.....	" = 1/8	60	10.2	9.8	−7
V.....	" = 1/3	60	10.5	10.1	−7
VI.....	" = 1/8	90	10.5	10.3	−4
VII.....	" = 1/20	120	11.0	10.9	−2
VIII.....	" = 1/8	150	9.9	9.8	−2
IX.....	" = 1/5	150	10.6	10.4	−4
IX.....	" = 1/5	150	10.6	10.5	−2
X.....	" = 1/8	180	10.5	10.4	−2
XI.....	" = 1/2	180	10.6	10.5	−2
XI.....	" = 1/2	180	10.7	10.4	−5

order to prevent the loss of good material through premature shedding of the eggs and sperm.[6]

With these precautions the titration of the chlorides yields perfectly consistent results.

Considering the sources of error and especially the difficulty of measuring the volume of a large number of eggs, the constancy in the sense of these differences is impressive. There is unquestionably a chlorine-deficit in egg-secretion.

IV. The Origin of the Chlorine-Deficit.

How does the chlorine deficiency arise? There is a presumption in favor of attributing it to the eggs; on the other hand, if these eliminate substances capable of masking the chlorine, $AgNO_3$ would give no more clue to its presence than in the titration of chloroform or trichloracetic acid. The problem is soluble by two very simple tests.

If the chlorine is removed by the eggs rather than masked by the exudates, it should be possible to prepare egg-secretions without a chlorine-deficit. To accomplish this the eggs should be exposed to sea-water until all the chlorine which they are able to hold has presumably been taken up. Such eggs if subsequently permitted to secrete into a fresh volume of sea-water should remove no chlorine whatever on their second exposure.

The reasoning is justified by the following experiment in which 1 c.c. of control sea-water, 1 c.c. of first sea-water and 1 c.c. of second, are all expressed in terms of $AgNO_3$ *n*/20.

Table III.

Ratio.	Time.	Control Sea-water.	1st Sea-water.	2d Sea-water.
$\frac{\text{Eggs}}{\text{Sea-water}} = 1/8$	11 A.M.	10.5	10.5	
	11:30	10.5	10.3 Eggs transferred to 2d Sea-water.	
	11:30			10.5
	12:00	10.5		10.5

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[6] The efficacy of a three- to five-minute submersal in fresh water was first noticed by my colleague, Miss Sampson.

This result however does not yet solve the problem. It might be argued that the substance which masks the chlorine is secreted only by eggs newly shed from the ovary. If true, the failure to remove chlorine in the above experiment from the second volume of sea-water could be attributed to the absence of the chlorine-masking secretion.

A direct test of this idea is easily made, for if the deficit were, in reality, only apparent, it should be possible by complete evaporation to recover from equal volumes of sea-water and of secretion, equal quantities of sea-salts. This, as the following comparison shows, is not the case.

Table IV.

	Chlorine per c.c. as $AgNO_3$ $n/20$.	Total Salt per c.c.
Sea Water	10.6 c.c.	.0387 gram
Secretion	10.4 c.c.	.0370 gram

We must conclude then that the chlorine is not masked by the organic materials in the secretion, but is removed from solution by the eggs. Moreover, if we arbitrarily assume that the eggs remove only KCl, which in relation to its chlorine content is the heaviest of the salts present, there would still remain a discrepancy between the total salt-deficit of 1.7 milligrams per c.c. of secretion and the loss attributable to KCl alone. The total deficit therefore does not appear to result from a selective action on the part of the eggs. On the contrary, we must believe that all the salts are affected in proportion to their concentration in sea-water and their capacity for being removed by the particular egg-mechanism involved in the process.

V. The Mechanism by which Salts are Removed from the Sea-Water.

Although an inspection of Table II. suggests that the chlorine deficit does not increase after the first fifteen minutes of exposure, the actual state of affairs can be rendered much clearer by reducing the values given to a common basis. We will assume an ample supply of chlorine; also, for the sake of comparison, that in one hour, 1 c.c. of eggs can remove from 1 c.c. of sea-water as much chlorine as from twenty; and further, that in 1 hour 1 c.c. of eggs removes one third as much as in three. On the basis of these assumptions and the actual titrations, we can construct a curve showing the comparative amounts of chlorine which, under the conditions imagined, 1 c.c. of eggs would abstract from 1 c.c. of sea-water in one hour, if the rate of removal for that hour were constantly the rate deducible from the determinations actually made after exposures of 15, 60, 90, 120, 150, and 180 minutes.

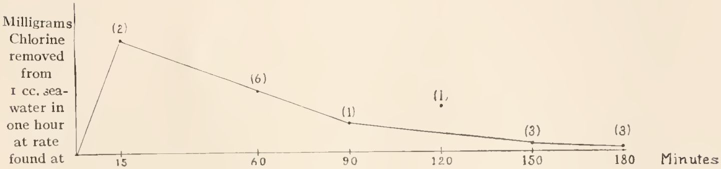

Fig. 1. Curve showing gradual loss in the power of eggs to abstract salts from sea-water.

The number of titrations upon which the points indicated in the curve rest, is given in brackets. Clearly there is a steady approach to zero. Since the rate of chlorine removal by eggs immersed for 180 minutes falls from the height which it had reached after 15 minutes it follows that the process of salt-abstraction is reversible. However, there is no equilibrium. We must suppose therefore that the chlorine-removing mechanism slowly breaks down.

Within the time-limit of these experiments, the only part of the egg-system known to undergo dissolution is the jelly which surrounds each newly shed egg. This chorion disintegrates slowly in sea-water. Fortunately it can also be readily removed by moderate shaking. I therefore compared the chlorine deficits produced by equal volumes of normal and dechorionized eggs in equal volumes of sea-water.

The experiment, of course, involves two errors: in the first place one cannot be certain that the jelly has actually been removed from every egg; and secondly, its removal from a considerable number renders it certain that a given volume of dechorionized ova will contain a larger number of cells than an equal volume of normal eggs. Both errors really operate against any à priori idea that the jelly is responsible for the removal of the salts. If then the result is clear cut nevertheless, it would appear that the experiment is decisive.

Two such comparisons are given in Table V.

Table V.

Chlorine per c.c. in Terms of $AgNO_3$ $n/20$.

	Sea-Water Control.	Sea-Water with Normal Eggs.	Sea-Water with Chorion-free Eggs.
After 30 minutes	11.0	$\frac{\text{eggs}}{\text{sea-water}}\ \frac{.2\text{ c.c.}}{14.8\text{ c.c.}} = 10.9$	$\frac{\text{eggs}}{\text{sea-water}}\ \frac{.2\text{ c.c.}}{14.8\text{ c.c.}} = 11.0$
After 2 hours	11.0	$\frac{\text{eggs}}{\text{sea-water}}\ \frac{.2\text{ c.c.}}{10\text{ c.c.}} = 10.9$	$\frac{\text{eggs}}{\text{sea-water}}\ \frac{.2\text{ c.c.}}{10\text{ c.c.}} = 11.0$

A further test seemed desirable. If the jelly takes up the salts, it should be possible to demonstrate their presence in the chorion. The method was that of Macallum[7] in which the reagent, $n/10$ $AgNO_3$ is acidulated, per liter, with 25 c.c. of 60 per cent. HNO_3 in order to avoid any confusion that might result from possible phosphate precipitates or combinations of the silver with proteins or their constituent parts.

The eggs after being carefully drained were gradually transferred to absolute alcohol, and after hardening, subsequently treated with Macallum's reagent for half an hour. After this they were placed on a glass slide, cleared in glycerine under a cover slip, and exposed to direct sunlight for 30 minutes. The distribution of the reduced silver is shown in Fig. 2.

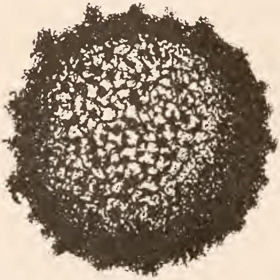

Fig. 2. *Arbacia* egg with chlorides indicated in chorion.

[7] Macallum, A. B., Die Methoden der Biologischen Mikrochemie. Abderhalden's Handb. d. Bio-chem. Arbeitsmethoden, 1912, p. 1100.

Conclusions.

Any attempt to standardize *Arbacia* egg-secretion by physical methods must take into account the fact that these eggs temporarily lower the specific gravity of sea-water. This renders unreliable any immediate application of methods depending on surface tension, viscosity, or specific gravity.

The decrease in density is accompanied by a measureable chlorine deficiency in the solution and by a greater shortage of total salt. We cannot attribute these effects directly or indirectly to the substances which the eggs eliminate since a preliminary exposure of the eggs to sea-water enables us to produce on second exposure a secretion without salt-deficit. Moreover, the results of total evaporation show that the salts were definitely out of the solution and that there is no selective abstraction by the eggs other than that dependent on the proportions in which the several salts are present and their capacity for being removed by the particular egg-mechanism which is involved in the process.

This mechanism is the chorion, for eggs deprived of their jelly by shaking do not cause a salt-deficit. Localization by means of $AgNO_3$ $n/10$ in the presence of HNO_3 demonstrates the concentration of chlorides about the eggs.

The concentration is temporary since the chorion within a few hours normally undergoes disintegration in sea-water. As the result of this the salts are dispersed and the specific gravity of the sea-water may return to normal. A rise above normal may be attributed to the presence of exudate in solution.

In time these facts may find an application in the theory of fertilization. For the present they are presented without theoretical bias although there are unavoidable suggestions in the fact that the concentration of sea-salts immediately about an *Arbacia* egg is temporarily and measurably greater than the concentration of the same salts in the surrounding sea-water.

March 1, 1922.

NOTES ON THE OCCURRENCE OF ABNORMAL MITOSES IN SPERMATOGENESIS.

ROBERT H. BOWEN.[1]

Mitotic figures abnormal particularly with respect to the number of chromosomes and centrioles involved are, as is well known, of common occurrence in the spermatogenesis of many insects. Apart from their interest as mere abnormalities, these cases sometimes throw light on more important problems of cell division and acquire, therefore, a much greater significance. In the course of several years' study of hemipteran (Family *Pentatomidæ*) spermatogenesis a variety of abnormal types of division have been observed, and it has seemed worth while to assemble descriptions of some of these, in particular those not hitherto described by other workers. The cases here reported are all from the male germ cells of various Hemiptera, belonging with one exception to the Family *Pentatomidæ*. The material was originally fixed for a variety of different purposes and the methods are accordingly noted separately where necessary. I am indebted to Professor E. B. Wilson for the use of a number of slides of *Loxa florida* which he had prepared some years ago and which I found presented a very unusual type of cell division.

Abnormal mitoses occur sometimes as solitary exceptions among many normal cells, but at other times they occur in large numbers in a single testis. In the latter case the abnormal cells may form a large percentage of the cells in a single spermatic cyst. Frequently the condition which produces these irregularities is more widely operative with the result that large numbers of cells in a given testicular lobe or follicle are involved. The isolated cases are doubtless due to occasional 'accidents' such as are to be expected in all vital phenomena; but the simultaneous occurrence of many abnormal divisions seems sometimes to depend on other causes, the nature of which is for the most part unknown.

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[1] From the Department of Zoölogy, Columbia University.

There are obviously three possible types of abnormal cell division, which may be outlined as follows:

Case 1.—The number of chromosomes is normal, but the number of centrioles is abnormal.

Case 2.—The number of centrioles is normal, but the number of chromosomes is abnormal.

Case 3.—The number of both chromosomes and centrioles is abnormal.

Examples of these three cases will be considered in order.

CASE I.

The occurrence of dividing cells in which a normal number of chromosomes is arranged on a multipolar spindle is very rare. Indeed, I have found only a single case of this kind, and do not recall having noted any similar cases in the published work of other writers. This unusual example was found in a testis of *Chelinidea vittiger* Uhl. (Family *Coreidæ*), which did not present any other unusual features. In the male of *Chelinidea* the spermatogonial chromosomes number twenty-one, of which two are very small—so-called *m* chromosomes—and one, an unpaired *X* chromosome. In the first maturation division the chromosomes, eleven in number, are arranged in the manner characteristic of other coreids.

The abnormal division here reported occurs in a cyst of spermatocytes in the metaphase of the first maturation division. The spindle is tri-polar (Fig. 3) and the centriole at each pole can be distinctly identified. The legs of the triangular spindle figure are nearly but not quite equal; otherwise it is very regular in formation. The eleven chromosomes characteristic of this division can be easily counted, and in most cases their distribution to the spindle poles can be foretold. Their arrangement on the spindle is very regular, and it will be readily seen that each pole of the spindle will receive approximately the same number of anaphase chromosomes. In the figure the chromosomes are numbered according to the approximate level of focus at which they appear, No. 1 being the topmost (the two topmost chromosomes (No. 1) were added from a contiguous section).

The disposition of the *m* chromosomes is particularly interesting. Ordinarily they conjugate very briefly on the spindle (Wilson, '05*b*), and then separate precociously; but in this case they seem to have gone on the spindle entirely separate from each other (even their customary semblance of synapsis has failed). One gets the impression that they receive spindle fiber attachments at both ends, although a single one might be expected if such attachments are invariably specific.

The origin of the third centriole in a case of this kind is obscure, but it is possible that a tripolar spindle might arise through the precocious separation of one of the pairs into which the centrioles of the hemiptera are characteristically divided at an early stage in the growth period of the primary spermatocytes; but the staining of the centrioles in the case which I have described was not sufficiently precise to permit analysis of this possibility.

CASE 2.

Cases in which normal, bipolar spindles are developed in connection with an unusual number of chromosomes are of not infrequent occurrence. The best known examples are to be found in triploid and tetraploid "mutants," in which the abnormal chromosome number exists in all the cells of the body. Similar multiplications of the normal chromosomal complex likewise occur in the germ cells of normal individuals with some frequency, either as isolated cases or in groups. Henking ('91), who first made a critical examination of hemipteran chromosomes (in *Pyrrhocoris*), seems to have noted a case of the occurrence of the diploid chromosome number in the first maturation division (Henking ('91), Fig. 30*a*), though his explanation of the make-up of the doubled chromosomes now seems doubtful. He noted also the occurrence of a tetraploid connective tissue cell, an abnormal condition commonly (if not constantly) present in the cells which form the sheath of the hemipteran testis and its smaller subdivisions. Hartman ('13) has more recently reported several cases in the spermatogonia and primary spermatocytes of the grasshopper (*Schistocerca* and *Melanoplus*) in which extraordinary chromosome numbers were encountered. Morgan ('15) has reported a case in *Phyllaphis* which differs from the above in that a considerable number of cells are affected rather than a few isolated ones. In this particular instance all the cells of an entire cyst (male) contained the double number of chromosomes, which were readily counted, as the cells were just concluding the first spermatocyte division. This case presents one important point of difference from all other examples of abnormal division in that "the chromosomes are only half as large as are those at the corresponding stage of the normal spermatocyte stage"—a very unusual abnormality indeed.

In the material which I have examined two instances of abnormal chromosome number have been found which resemble the one described by Morgan in that large numbers of cells are affected, *but* the chromosomes are *not* reduced in size. A description of these cases will now be given.

The first, and in some ways the less interesting, of these cases was found in a specimen of *Loxa florida* Van D. (Family *Pentatomidæ*), of which both testes were affected in exactly the same way and to the same extent. This particular abnormality was restricted to a single lobe of the testis which happens to be composed of cells of an unusually small size (see Bowen '22).[1] The earlier stages in the affected lobe seem to be normal in every way (Fig. 4), but with the inauguration of the growth period of the primary spermatocytes a very unusual phenomenon sets in. Large numbers (but not all) of the cells in each cyst fuse together in pairs to form giant, bi-nucleate cells (Fig. 5). The cytoplasmic masses of the two cells seem to fuse completely, but the nuclei remain separate although they frequently become so closely appressed as to show little visible separation. As the growth period proceeds, this process of fusion goes on progressively, though somewhat irregularly, with the result that eventually the prophase cysts contain a heterogeneous mass of cells possessing in individual cases anywhere from one to eight or more nuclei (Fig. 6), each with its own complement of chromosomes.[2] The cells round out and become somewhat separated from each other, those containing numerous nuclei being roughly spherical in shape and of extraordinary size. The nuclei themselves become more or less lobulated during the growth period and so are difficult to separate and count, but the grouping of the late prophase chromosomes shows that each nucleus retains its identity and that the fusion process involves only the cytoplasms of the uniting cells. The remarkable fact about these giant cells is that they seem to possess only the single set of centrioles characteristic of a normal cell. Often these are not diametrically opposite each other (as is customary in Hemiptera), and as the spindle is formed for the first maturation division it tends to develop toward one side of the cell, becoming later on symmetrically located. How the centrioles of each of the cells represented in one of these giant spermatocytes are reduced to a normal number is not known; possibly the loss occurs before the fusion, which might itself be dependent on such an abnormal condition. Further, the centrioles which do take part in the formation of the spindle, are not unusually large, as one might anticipate, but are in fact no larger than the normal ones, at least not conspicuously so. Spindles are normally developed, and the metaphase chromosome plates are formed in a characteristic manner (though slightly irregular in cells with large numbers of chromosomes). I have been able to count cases with 16 and about 32 chromosomes, the higher numbers being less satisfactory for counting. I have, however, counted considerably more than 60 chromosomes in a single plate. Second maturation spindles seem to be normally formed (except, of course, with respect to chromosome number) and spermatids of a variety of sizes result, each spermatid being formed on a large scale but in an otherwise normal fashion. Whether the giant sperms resulting from the multinucleate spermatocytes reach maturity was not definitely ascertained, but at least some of them seemed to be undergoing the earlier stages of normal differentiation.

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[1] I have been able to examine only a single specimen of this species. It would be interesting to find out whether this abnormality has any relation to the sizes of the cells which might possibly cause its production repeatedly.

[2] In *Loxa* the spindle of the first spermatocyte division normally has eight chromosomes, as in a number of other pentatomids (*e.g.*, *Euschistus*).

The origin of this abnormality is obscure. However, the apparent fusion (?) of cells to form multinucleate masses is not altogether unknown, as such aggregates have been noticed by Wodsedalek[1] in a number of mammals, particularly the ground squirrel (*Citellus*). In the last-mentioned case, the spermatids also are multinucleate, which would seem to preclude the possibility of their forming normal sperms—a point in which this case is quite different from the one described in *Loxa*.

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[1] Only a very brief abstract of this author's results has been available; see Abstracts of Papers, American Society of Zoölogists, Nineteenth Annual Meeting, December, 1921.

The second instance of abnormal divisions falling under *Case 2* presents in many ways the most interesting features of all. It is clear that aside from the method just described of multiplying chromosome numbers by cell fusion, the same result might be produced by some failure in spermatogonial division, such that the daughter chromosomes would be incorporated in a single nucleus, while the extra set of centrioles in some way became suppressed or lost. Such might conceivably have been the genesis of the case described by Morgan ('15), and of various triploid and tetraploid 'mutations.' The case now to be described seems to belong in this category. It was found in a specimen of *Euschistus variolarius* P. B. (Family *Pentatomidæ*), which had been collected in the fall of 1918 by Professor Wilson (without of course, any suspicion that it was abnormal), and kept in the laboratory during the winter. Upon my return to Columbia in February, 1919, this bug, together with other specimens of *Euschistus,* was very kindly turned over to me by Professor Wilson. The abnormality was found in only one specimen (No. 94) (of those sectioned), and in only *one* testis of that specimen. The other testis was normal in respect to this particular irregularity, though both presented other abnormal features. Whether the stay in the laboratory under somewhat unfavorable conditions was in any way responsible for the abnormalities is by no means clear; but from the fundamental nature of the particular abnormality here to be considered, it would seem more probable that it arose during one of the earlier instars and was quite uninfluenced by the abnormal environment of the adult. The material happens to have been fixed in Benda's Flemming (with the omission of the mordanting customary in the Benda method itself), and stained in Fe-hematoxylin. This method, intended primarily for the demonstration of the mitochondria, is not entirely satisfactory for the study of chromatic features; but the preparations proved entirely adequate for the study of most of the necessary details.

This particular abnormality consists of a tetraploid chromosome number in most of the cells of one of the large-celled lobes (lobe No. 4—Bowen, '22) of one testis. There are six lobes in the testis of *Euschistus* (Bowen, '22), and in this particular testis I have been able to make counts of spermatogonial metaphase chromosome plates in five of the lobes. In four of these (lobes No. 2, 3, 5 and 6—Bowen, '22) the number of chromosomes is 14 (normal for the genus (Montgomery, '11)), and it is clear from other evidence that the one lobe (No. 1) in which no spermatogonial divisions could be counted, is likewise normal in chromosome number. In one lobe (No. 4), however, the chromosomes are very clearly abnormal in number as could be readily told from numerous metaphase plates. The exact number was not easily arrived at because the plates tend to be a trifle irregular, but in one or two particularly good cases I have been able to count 28 chromosomes with satisfactory clearness (Fig. 7). In adjacent cysts of spermatogonia in which the nuclei are in early prophase stages it can also be easily seen that the chromosomes are present in abnormal number, though exact counts were of course impossible. In the growth stages of the spermatocytes and in the spermatid stages the double number of chromosomes can be surmised from the unusually large size of the cells, which increase is shared by the cytoplasm as well as the nucleus. The tetraploid condition is, therefore, present throughout the entire lobe, though not exclusively, for I was able to find an occasional cyst (growth period and spermatid) in which, judging from the comparative sizes of the cells, the chromosomes were present in the normal number. These facts lead me to suppose that an irregular division of a germ cell at some very early stage gave rise to an abnormal series of spermatogonial cells which formed the bulk of the cells in this particular lobe. Among these, however, were included a few normal cells which evidently gave rise to the normal cysts found scattered infrequently among the abnormal ones.

Analysis of the constitution of the metaphase spermatogonial plates with 28 chromosomes should bear out the view that the tetraploid chromosome number represents a simple doubling of the usual diploid group. Unfortunately, the chromosomes in *Euschistus* are not of strikingly different sizes, but in the case of the *Y* chromosome the difference is sufficiently marked to be readily distinguished; and as examination of Fig. 7 will show, there are two *Y* chromosomes (the very small ones) instead of the usual one (compare Montgomery, '11, Fig. 2). It is, I think, fair to assume that the other chromosomes are similarly represented in a duplex manner, an assumption which accords with the view now generally accepted of the nature of tetraploid chromosome groups.

Synapsis is apparently accomplished in a normal manner, and after the "diplotene" threads have become spread throughout the nucleus it is possible, in favorable places, to see that the threads are arranged in *pairs* and not in *quartets* as one might conceivably expect. In other words the homologous chromosomes are paired off just as they would have been in the presence of the diploid chromosome number, except that in each tetraploid nucleus there are two similar pairs instead of one. During the so-called confused period, when the chromatin threads become indistinct, the sex chromosome nucleoli stand out with great clearness. We should expect in a tetraploid nucleus four such nucleoli (barring possible fusions such as sometimes normally occur), representing two *X* (larger) and two *Y* (smaller) chromosomes. This expectation is exactly realized (Fig. 8), another bit of evidence which tends to prove that we are dealing here with a case of true tetraploidy.

When the chromosome tetrads condense in the prophases of the first maturation division, they resemble in every way the tetrads of the normal spermatocytes. (Compare Fig. 9 with Montgomery ('11), Figs. 86, 88 and 93.) It is clear beyond question that homologous chromosomes have paired off in a perfectly normal manner. These tetrads become condensed in the usual way to form the definitive chromosomes of the first maturation division, and the spindle of this division, when seen in side view (Fig. 10), presents no unusual features (except of course for the chromosome number). There happened to be one cyst of cells in various phases of the first spermatocyte division and these dividing cells have been carefully studied. I have been able to find nothing in the manner of spindle formation or chromosome division in any way abnormal, except that the plates have the diploid rather than the haploid number of chromosomes. This point was studied with special care. Two metaphase plates in polar view were found in which the chromosomes could be readily counted, although both were a trifle irregular in arrangement. One of these plates (Fig. 11) seemed to be in the final stage of formation, and some of the chromosomes were still not completely oriented. Fortunately this was particularly true of the *Y* chromosomes, whose constitution was thus rigorously demonstrated. In the normal cells the *Y* chromosome is represented by a dyad which is divided in the first spermatocyte division (see Montgomery, '11, Fig. 94). In a tetraploid cell we should expect to find two such dyads, and this is actually found to be the case. The *Y* chromosomes have not conjugated, but each has behaved exactly as though the other were absent. The parts of the dyad have been in each case precociously separated (Fig. 11). Furthermore, two unusually large chromosomes can be readily picked out (*A* and *A'*), and these seem to correspond to the single large chromosome of the normal haploid plate (see Wilson, '05*a*, Fig. 3*a*). A count of all the chromosomes (counting the *Y* dyads each as a single chromosome) gives a total of 17—one more than the expected number (16, or 8 times 2). It seems likely that the extra chromosome may be due to the counting of an *X* dyad, the parts of which have become separated, as two chromosomes instead of a single one. In Fig. 12 another plate is given which, judging from its neighbors, is probably in a very early anaphase stage. The plate is somewhat more compact, and the *Y* chromosomes, now presumably being drawn apart, are seen as single chromosomes. In this plate 16 chromosomes are clearly to be counted, although one of them is irregularly placed.

Unfortunately no second maturation divisions were found, so that it was impossible to check up the behavior of the *X* and *Y* chromosomes in that division. The spermatids seem to be normal in every way, except that they are larger than is usual; and at least the earlier stages of differentiation are gone through in a normal manner. Subsequently, some of them certainly become abnormal, but so do some of the sperm in an adjacent lobe (No. 6); and it is, therefore, impossible to tell whether the degeneration is connected with the tetraploid condition or not.

CASE 3.

The occurrence of divisions with abnormal numbers of both chromosomes and centrioles is of common occurrence in the maturation divisions in Hemiptera (see for example Henking, '91), and such divisions have also been described in spermatogonia (Montgomery, '98), and in cells from the connective tissue of the testis (Paulmier, '99). The general features of these divisions are well known, and they probably originate from abnormal spermatogonial divisions in which the daughter chromosome groups have failed to produce separate nuclei. In the cases here noted, from *Chlorochroa uhleri* (= *persimilis*) Stål (Family *Pentatomidæ*), large numbers of cells were affected particularly along one half of one testicular lobe, and to a lesser degree the contiguous portion of an adjacent lobe. In some cysts, as could be told from the unusual size of the nuclei with extra chromosomes, large numbers of spermatocytes (and spermatids) were affected, while in others all the cells were normal. The causative agent operative in this case would accordingly seem to differ from the more or less accidental sources of abnormal division figures. The chief interest of this case lies in the fact that the material had been prepared to demonstrate the Golgi apparatus, and it was accordingly possible to study the distribution of the dictyosomes (fragments of the Golgi apparatus) in relation to the multipolar spindle.

Numerous cases of tripolar[1] spindles in the first maturation division were found at both the metaphase (Fig. 2) and late anaphase (Fig. 1) stages, of which two are figured. As I have shown in another place (Bowen, '20), the dictyosomes collect (in equal amounts) around the ends of the normal (bipolar) spindle at the beginning of the metaphase, a position which they maintain during the anaphase, the Golgi material being thus distributed with approximate equality to the daughter cells. I suggested that the centrioles represent the morphological foci of the influences which bring about this equal distribution of the Golgi material.

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[1] There were also numerous cases in which the centriole number was normal, but with abnormal chromosome numbers, as in Case 2; but as these presented no points of special interest, they are not here considered. The distribution of the dictyosomes was in accordance with the expectation for a bipolar spindle.

If this suggestion be correct, in tripolar spindles the dictyosomes should obviously be arranged around each spindle pole (centriole) in a manner similar to that found in bipolar figures. This, is, in fact, the case as the figures show very clearly (Figs. 1 and 2), and seems to contribute additional evidence to that already accumulated tending to demonstrate an element of unusual regularity in the distribution of the Golgi apparatus in cell division.

Discussion.

Abnormal mitoses in the male germ cell cycle are chiefly of interest from the standpoint of the sperms which might be produced as a result of such divisions. It is clear that the sperms derived from divisions belonging to *Case 1* would always be abnormal; and those of *Case 3* (if the division were ever completed, which seems doubtful[1]) would likewise be abnormal in the great majority of cases. Whether any such abnormal sperms ever take part in successful fertilization is questionable. After divisions of the *Case 2* variety, however, there is no reason for supposing that the resultant sperms might not behave in an entirely normal manner in fertilization. This is particularly true of the case which I have described in *Euschistus,* and to the possibilities there presented I should like to give particular attention.

It is evident that tetraploid spermatogonia would probably give rise to diploid primary spermatocytes, and this probability has been definitely proved in the *Euschistus* case. It seems equally probable that the resultant sperms would likewise contain a group of chromosomes in the diploid rather than the normal, haploid number. Unfortunately definite proof of this has not been obtainable, but everything indicates that such is actually the case. If such diploid sperms should fertilize a normal (haploid) egg, a triploid individual would result; and if, as an almost impossibly rare coincidence, the egg should also be diploid, through some abnormality, a tetraploid individual would result. The triploid individuals might be numerous due to the involvement of a large number of sperms, but they would be accompanied by diploid individuals derived through some of the normal, haploid sperms which are produced by the normal portions of the testis. It is interesting to note that Bridges ('21) has recently reported a case of triploidy in *Drosophila* which exactly fulfills these expectations. The egg rather than the sperm, as could be determined from genetic evidence, happens, however, to have been the gamete affected, but the arrangement of the ovary is such that the difference in sex does not affect the applicability of the argument. The facts which I have been able to make out in *Euschistus* prove (so far as they go) that the presence of the chromosomes in the tetraploid number, following some irregularity of unknown origin, in no way affects the normal progress of spermatogenesis[1] (and presumably of oögenesis as well). This is, I believe, the first cytological evidence that abnormal germ cells, once established, may thereafter proceed in an entirely normal manner—probably with the ultimate production of functional sperms (or eggs).

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[1] It is perhaps more probable that these abnormal mitoses fail, giving rise to the familiar giant spermatids with one large nucleus but a multiple set of centrioles.

These facts have a very interesting bearing on the origin of triploid and tetraploid individuals, a matter in regard to which opinion is at present in a very unsettled state. It has been held by Gates ('09), with the subsequent assent of Strasburger, that the doubling of the chromosome number (tetraploid individuals) might arise "as the result of a suspended mitosis in the fertilized egg or in an early division of the young embryo." Stomps ('12), on the other hand, has suggested that tetraploid individuals may result from the union of two diploid gametes, triploid individuals being obviously formed on this hypothesis by the union of a haploid with a diploid gamete.[2] Further, the nature of the chromosome group in a diploid gamete is again open to a difference of opinion, since it is evident that the diploid number may have arisen from a normal (diploid) auxocyte through failure of reduction, or from a tetraploid auxocyte after normal reduction has occurred.

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[1] Provided of course that other disturbing elements such as multiple centrioles are absent.

[2] The possibilities of polyspermy have also been considered by Gates particularly in cases of triploidy, but our knowledge of this condition in animals lends no support to the belief that it is ever a factor in the production of triploid or tetraploid individuals. Further, both these authors were interested almost exclusively in plants (in which (*Œnothera*, for example) triploid and tetraploid individuals have actually arisen under observation), in which further special possibilities (apogamy, for example) have to be considered that are not met with in animals. These matters need not be considered further in this paper.

My observations indicate that the requisite conditions for the origin of the triploid, and perhaps tetraploid, individuals may be fulfilled by a combination of the possibilities suggested by the hypotheses outlined above. Thus, it seems certain in the *Euschistus* case that a suspended (or otherwise abnormal[1]) mitosis has occurred during some early division in the germ cells, giving rise to a tetraploid condition confined to the descendants of the particular cell involved. If, as seems probable, each cyst of spermatogonia is derived by the repeated division of a *single* cell derived from an early germ cell, the occurrence of the tetraploid cysts would be accounted for, and also the diploid cysts produced concurrently from the early germ cells of normal constitution. The spermatocytes descended from the tetraploid cells have undergone a *reduction* in the proper sense of the term, but the *number* of chromosomes in the resultant gametes is undiminished ("unreduced") as compared with the number in the normal (haploid) ones. The fusion of such a diploid gamete with a normal one would obviously produce a triploid individual. An explanation along these lines seems to me preferable to the one sometimes given which explains triploidy as due to the union of a normal and an unreduced (properly speaking) gamete; for while such unreduced gametes have been supposed to arise (in Hemiptera) through the suppression of one (or both) of the maturation divisions, they always contain an abnormal number of centrioles (as Paulmier, '99, long ago showed), and the weight of evidence is against the probability of their functioning normally in fertilization.

The origin of tetraploid individuals by this method would depend upon an exceedingly rare coincidence, and, as a matter of fact, tetraploidy in animals seems to be a rather rare phenomenon.

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[1] The case of *Loxa* suggests the possibility that a tetraploid germ cell might arise from the fusion of two normal ones. By analogy such a fusion product might well contain the normal centriole content, as observed in *Euschistus*. The usual explanations advanced to account for the doubling of chromosomes all fail to account for the observed normal number of centrioles—an important part of the story, which has not yet received adequate attention. In this connection it is of interest to note that centrioles are generally absent in the higher plants.

In plants, on the other hand, it occurs more commonly—in *Œnothera* for example, where the repeated occurrence of unusual chromosome complexes would perhaps indicate that some unstable condition exists which would increase the probability of a coincident production of diploid gametes of both sexes.

The viewpoint which I have here tried to develop may be summarized in the following way. A normal, diploid zygote is formed by the union of two haploid gametes.[1] After a number of divisions the germ cells (one or more?) are segregated, and these then multiply to form the primordial germ cells and subsequently spermatogonia (or oögonia). An abnormal division at some early stage resulting in a doubling of the chromosomes might conceivably give rise to a gonad composed entirely of tetraploid cells—a condition never yet observed. A similar abnormal division in one of the primordial germ cells would result in many cysts of tetraploid spermatogonia (or oögonia), as I have found in the *Euschistus* case—certainly a rare phenomenon or it would have been long since reported. Proceeding still further, a similar abnormal division at the time a cyst of spermatogonia (or oögonia) is begun, would give rise to a single cyst of tetraploid cells (see the case reported by Morgan, '15), while abnormal divisions at a subsequent time might give rise to one or several tetraploid cells among many normal ones in the same cyst. The tetraploid cells thus produced undergo normal *synapsis* and *reduction,* but the chromosome number, being thus diminished only by the usual one half, is diploid in the resulting gametes. The union of such a diploid gamete with a normal (haploid) one produces a triploid individual, while the union of two such diploid gametes (due to a very rare coincidence) produces a tetraploid individual.

In conclusion, it may be noted that the behavior of the chromosomes in the *Euschistus* case adds further evidence to that already accumulated tending to demonstrate the genetic continuity of the chromosomes. In the same testis there are (normal) diploid and (abnormal) tetraploid spermatogonia, each of which gives rise to the expected haploid and diploid chromosome complexes in the maturation divisions. The fact that in synapsis (and subsequent stages) the homologous pairs of chromosomes behave exactly as though they were present in the diploid number, is another fact of great theoretical significance. This last-mentioned point is of particular interest because of the condition recently reported in a triploid specimen of *Canna* by Belling ('21). This author finds that in the pollen-mother-cells there are nine *triads* (the haploid number is nine), "each of which separates into two and one on the spindle, in a random manner with regard to the two poles." In the tetraploid individual of *Euschistus,* on the other hand, no indication of a tendency to form multiple groupings of this kind has been observed at any stage.

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[1] It is conceivable that, as Gates suggests, the first (or other early) cleavage might be suppressed, resulting in a tetraploid individual, but there is no evidence among animals that this ever occurs, and the difficulties in the way of such an explanation are great.

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EXPLANATION OF PLATES.

All of the figures have been outlined as far as possible with the camera lucida at an initial enlargement of approximately 3,800 diameters. The outlines have been extensively corrected and details added free hand. In reproducing, the figures have been reduced uniformly to an enlargement of approximately 3,000 diameters. In every case the method employed in the preparation of the original object has been indicated.

P, plasmosome; *X* and *Y*, idiochromosomes.

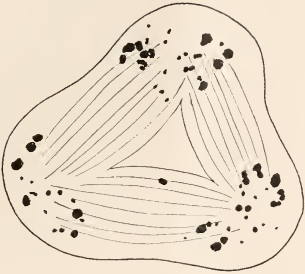
Plate I.

Figs. 1 and 2 are from *Chlorochroa uhleri;* Fig. 3 is from *Chelinidea vittiger;* Figs. 4, 5 and 6 are from *Loxa florida.* With the exception of 1 and 2, the original preparations were from material fixed in Flemming and stained with Fe-hematoxylin.

Figs. 1 and 2. Tripolar spindles in the first spermatocyte divison. Some of the chromosomes (stippled) are indicated in Fig. 2. (Osmic impregnation.)

Fig. 3. Tripolar spindle with normal number of chromosomes in the first spermatocyte division.

Figs. 4, 5 and 6. Primary spermatocytes in the later diplotene stage and in the earlier and later growth period, showing progressive fusion of the cytosomes.



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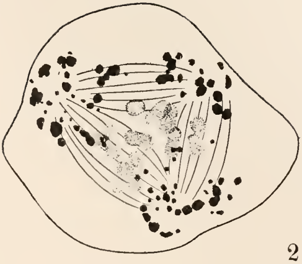

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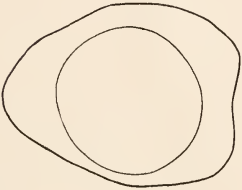

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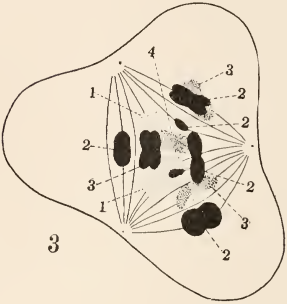
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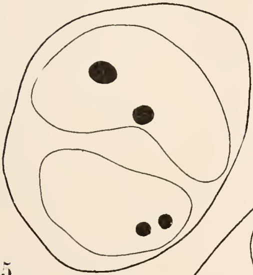

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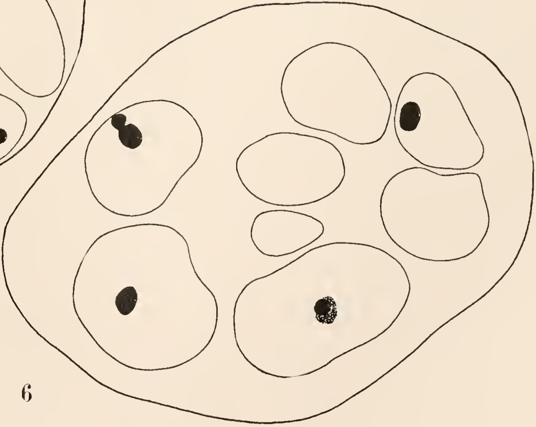

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PLATE II.

All the figures are from *Euschistus variolarius.* The original preparation was from material fixed in Benda's Flemming and stained with Fe-hematoxylin.

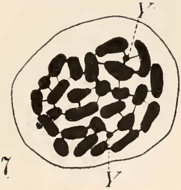
FIG. 7. Spermatogonial division (polar view) showing metaphase plate with 28 chromosomes.

FIG. 8. Primary spermatocyte in the later growth period.

FIG. 9. Single tetrad from a tetraploid spermatocyte nucleus in the late prophase.

FIG. 10. First spermatocyte divison; lateral view.

FIGS. 11 and 12. Early and late stages in the metaphase (approximately) of the first spermatocyte divison; polar view.



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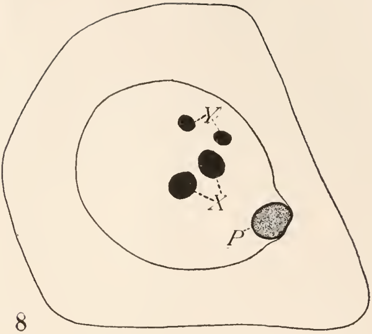

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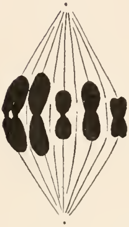

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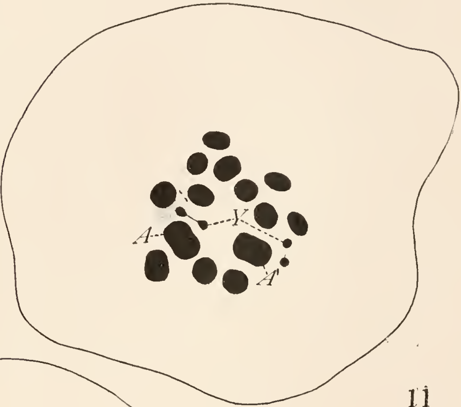

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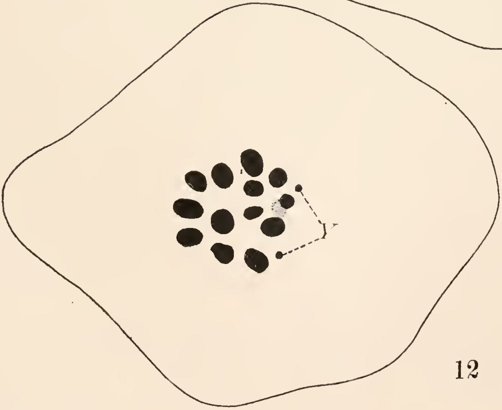

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ROBERT H. BOWEN.

RELATION BETWEEN INTENSITY OF LIGHT AND RATE OF LOCOMOTION IN *PHACUS PLEURONECTES* AND *EUGLENA GRACILIS* AND ITS BEARING ON ORIENTATION.

S. O. MAST AND MARY GOVER,

THE ZOÖLOGICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY.

INTRODUCTION.

According to the De Candolle-Verworn theory of orientation which has been widely accepted, the action of the locomotor appendages in organisms is dependent upon the intensity of the light received by the receptors connected with them; so that if one side of a bilaterally symmetrical organism, *Volvox, e.g.*, is more highly illuminated than the other the locomotor appendages on one side beat more effectively than those on the opposite side, resulting in turning until the two sides are equally illuminated and the organism is oriented. Torrey ('13), Bancroft ('13), Loeb ('18). and others maintain that orientation in asymmetrical organisms like *Phacus* and *Euglena* is in principle precisely the same as orientation in bilaterally symmetrical organisms. These organisms rotate on their longitudinal axes and proceed on a spiral course with a given surface continuously directed outward. The locomotor appendages are, consequently, on one side when the organism is in one position on the spiral and on the opposite side when it is in another position. Owing to this it is held by those mentioned above, that orientation in these asymmetrical organisms is essentially like that in bilaterally symmetrical organisms. If this contention holds it is evident that the rate of locomotion should depend upon the luminous intensity of the field. With this in mind we have investigated the relation between the intensity of light and the rate of locomotion in *Phacus* and *Euglena*.

Phacus pleuronectes.

Phacus is a small green flagellate somewhat like *Euglena* in structure and behavior. The species studied closely resembles

Phacus pleuronectes (Müller). It was kept in the laboratory in fairly good condition for several months. All of the specimens used in these observations were taken from the same culture jar. The experiments were all carried out in a large dark room. The apparatus used was arranged as follows: A rectangular box 180 cm. long, 15 cm. wide and 15 cm. deep, open at one end and the top, was placed on a table with a microscope near the open end. An electric lamp in the box was attached to the under surface of a short board which fitted into grooves on the top of the box, and could be moved back and forth so as to rapidly change the luminous intensity at the microscope. The lamp was so adjusted and screened that it produced a horizontal beam of light which crossed the stage of the microscope. The beam of light, before reaching the organisms, passed through 3 cm. of water in a glass container. Thus the longer waves from the lamp were absorbed by the water and any possible effect of heat on the behavior of the organisms was greatly reduced, if not entirely eliminated.

All of the observations were made under the low power of a compound microscope with the organisms on the stage in an aquarium, about 3 cm. square, constructed of the best plate glass obtainable. The eye-piece of the microscope contained a micrometer scale which divided the field into equal divisions, each .017 mm. long.

In making the observations, 30 to 40 specimens of *Phacus* were taken from a culture jar and put into the aquarium, which contained clear water about 1 cm. deep taken from the same jar. The aquarium was then put into the beam of light on the stage of the microscope and the distance from the lamp adjusted so as to produce the luminous intensity desired. An individual which was accurately oriented was now selected and carefully observed as it proceeded toward the light. (All of the observations were made on positive individuals.) When the anterior end reached a cross-bar of the micrometer scale the stop-watch was started, and when it reached the cross-bar 20 divisions, .34 mm., beyond, the watch was suddenly stopped and the time consumed recorded. The intensity was then suddenly raised or lowered and the same individual again timed, in the manner just described. Thus there were two readings made on each individual. In some cases the readings were made first in the higher, and in other cases first in the lower intensity. When the intensity was increased there was ordinarily no shock-reaction, and the organisms were timed immediately after the intensity was changed, *i.e.*, shortly after the close of the test in the lower intensity. But when it was decreased there usually was a shock-reaction, and if this occurred, the reading was not started until after the organisms had fully recovered which usually required only a few moments. The intervals preceding the readings in the lower intensities were, however, always somewhat longer than those preceding the readings in the higher intensities.

The results obtained are presented in Tables I. and II. Table

TABLE I.

RELATION BETWEEN INTENSITY OF LIGHT AND RATE OF LOCOMOTION IN *Phacus pleuronectes* (POSITIVE INDIVIDUALS).

Each line contains two readings for one individual in different intensities of light; the last line, the averages for all 20 individuals. The record in the lower intensity was obtained first in each instance.

Designation of individuals.	Time, in seconds, required to travel .34 mm. in an intensity of 1023 m.c.	Time, in seconds required to travel .34 mm. in an intensity of 4128 m.c.
1	5.7	6.2
2	5.4	5.8
3	6.7	7.4
4	5.5	5.2
5	6.4	7.0
6	7.0	7.5
7	6.2	6.2
8	7.0	7.2
9	4.9	4.4
10	6.0	5.7
11	5.5	5.4
12	5.4	5.3
13	5.4	5.3
14	5.0	4.7
15	5.2	5.2
16	4.8	4.8
17	5.0	5.2
18	4.7	4.9
19	5.2	5.0
20	5.7	5.5
Average	5.63	5.69

I. includes, for each of 20 individuals, two records, one taken in higher and the other in lower intensity. In each case the record for the lower intensity was obtained first. That is, the rate of locomotion for a given individual was ascertained in the lower intensity, then the light was moved nearer to the microscope and the rate ascertained for the same individual in the higher intensity. After the two readings had been obtained for one individual, the light was moved to its original position, another individual timed in the same way, and so on until the rates for 20 or more individuals were recorded.

By referring to Table I., it will be seen that the rate of locomotion of any one individual in the lower intensity was practically the same as it was in the higher intensity. The table shows that the average time required to travel .34 mm. was 5.63 sec. in the lower and 5.69 sec. in the higher intensity, indicating a slightly higher rate in the lower intensity. The difference referred to is, however, only .06 of a sec. This is probably within the limits of error, since a further examination of the table shows that the rate was not consistently higher in the lower intensity, 9 individuals traveling faster in the higher intensity, 8 slower and 3 at the same rate in the two intensities. That is, the rate varied about as frequently in one direction as in the other. This shows that there is, in *Phacus,* practically no difference in the rate of locomotion in luminous intensities varying from 1023 m.c. to 4128 m.c., and it indicates that locomotion is not to any considerable extent immediately related with the intensity of the illumination.

A summary of the results obtained in all of the observations made are presented in Table II. By referring to this table it will be seen that the rate of locomotion was on the average slightly higher in the lower intensities than it was in the higher, the average time required to travel 0.34 mm. being 6.002 seconds in the former and 6.134 seconds in the latter. The difference is, however, so small and inconsistent, being in favor of the higher illumination in two out of the nine sets of tests, that its significance is questionable. These results support the conclusions formulated above. They show that the rate of locomotion in *Phacus* is within wide ranges, practically independent of the intensity of light when exposed for short periods of time, and that if light has any immediate effect on the rate, it has a retarding effect.

TABLE II.

RELATION BETWEEN INTENSITY OF LIGHT AND RATE OF MOVEMENT IN *Phacus pleuronectes* (POSITIVE INDIVIDUALS).

Records in the higher intensity obtained first in the first three sets of experiments and last in the rest.

Each line gives the average rate, for a set of individuals, in a higher and a lower intensity. Each individual represented in the averages was timed twice, once in a higher, and once in a lower intensity. The + signs in the last column indicate a higher rate in higher intensity, the — signs a lower rate in the higher intensity.

Number of Individuals.	Intensity in m.c.	Average Time, in Seconds, Required to Travel .34 mm.	Intensity in m.c.	Average Time, in Seconds, Required to Travel .34 mm.	Difference between the Time Required in the Two Intensities.
15	91	5.71	325	5.73	−.02
20	106	6.90	325	6.55	+.35
20	58	6.58	325	6.96	−.38
12	91	5.26	325	5.36	−.1
13	31	5.91	325	5.82	+.09
20	31	5.78	325	6.01	−.23
20	16	5.44	325	6.05	−.61
20	1,032	5.63	4,128	5.69	−.06
18	459	6.81	4,128	7.04	−.13
Total average		6.002		6.134	

Euglena gracilis.

The observations on *Euglena* were all made on one species (*gracilis*). The specimens used were obtained in laboratory cultures containing wheat, a substance which is very favorable for the growth of this species. The methods employed were precisely the same as those employed in the study of *Phacus* with the following exceptions: The observations extended over a wider range of intensities; they were made under a binocular in place of a compound microscope; and each individual was tested successively in four different intensities beginning in every case with the lowest, in place of in two different intensities beginning sometimes in the higher and sometimes in the lower; and the course was 0.74 mm. in place of 0.34 mm. long.

A summary of the results obtained is presented in Table III.

Table III.

Relation Between Rate of Locomotion and Luminous Intensity in *Euglena gracilis.*

Each individual was timed successively, once in each of the intensities indicated, beginning in every instance with the lowest. The time presented is, in every case, the average of that required for each of the individuals indicated to travel once over the course in the illumination indicated.

Date of Observation.	No. of Individuals.	Time, in Sec., Required to Travel 0.74 mm. in an Intensity of			
		286 m.c.	459 m.c.	853 m.c.	2106 m.c.
Jan. 19...	8	5.88	6.05	5.53	5.12
Jan. 28...	2	5.15	5.6	5.65	5.75
Jan. 28...	2	6.1	6.5	5.55	5.95
Feb. 1....	2	4.7	4.7	3.6	4.9
Feb. 1....	8	7.47	7.55	7.91	7.64
Feb. 6....	8	6.01	5.36	5.17	5.27
Feb. 7....	26	5.7	5.36	5.30	5.45
	Average....	5.858	5.873	5.53	5.725
		84 m.c.	142 m.c.	286 m.c.	853 m.c.
Feb. 8....	2	5.85	6.1	5.9	5.5
Feb. 12...	7	8.8	8.45	8.82	8.73
	Average....	7.325	7.275	7.36	7.115

This table shows that in intensities ranging in the one set of experiments from 286 m.c. to 2106 m.c. and in the other from 84 m.c. to 853 m.c. there is remarkably little difference in the rate of locomotion, the difference between the rate in the lowest intensity and that in the highest intensity being only .0276 + mm. per second in the one set and .0298 + mm. in the other. It shows that, in the time it takes *Euglena* to travel 10 mm. in the lowest intensity it would travel only a little more than 10.2 mm. in the highest intensity, and that the lowest rate is not consistently in the lowest intensity.

The results obtained consequently indicate that light in certain intensities slightly accelerates locomotion in *Euglena,* and slightly retards it in *Phacus.* Orientation cannot, however, be due to the effect on the rate of locomotion of difference in the illumination of the sensitive tissue in different positions on the spiral course of these organisms, as is demanded by the De Candolle-Verworn theory applied to asymmetrical organisms by Torrey, Bancroft and others, for the effect on the rate is, even under the most favorable condition, so small that if orientation were dependent upon this, it would require much longer than it actually does; and moreover, orientation occurs under luminous conditions in which an increase or a decrease in intensity does not appear to appreciably effect the rate of locomotion.

The facts presented above have no bearing on the question as to the effect of light on the rate of locomotion in long exposures. All of the evidence obtained by various investigators in reference to this indicates that organisms like *Phacus* and *Euglena* come to rest if they are subjected for long periods to low illumination or darkness. This is probably owing to the effect of light on physiological processes (*e.g.*, photosynthesis) which in turn affect the activity of the organisms.

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DEDIFFERENTIATION IN ECHINUS LARVÆ, AND ITS RELATION TO METAMORPHOSIS.

J. S. HUXLEY,

New College, Oxford.

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1. Introduction.

The following observations and experiments were made at the Marine Biological Association's Laboratory at Plymouth in July and August, 1920.

The accidental discovery that dedifferentiation, of a type similar to that already studied in *Clavellina*, occurred in Echinoderm larvæ led me to make further observations on this phenomenon. Owing to lack of material, they are very fragmentary. I hope to resume them at the earliest opportunity. Meanwhile their theoretical bearing on the question of metamorphosis warrants their publication in their present form.

I have to thank the Director and Staff of the Plymouth Laboratory for their assistance. The work was carried on with the aid of a grant from the Royal Society.

2. Differential Modification of Growth.

Experiments were started on fertilized eggs of *Echinus miliaris* in order to test certain conclusions of Child's ('16 and '17) as to the effect of dilute poisons on differential modification of growth, and to compare the effects of cyanides and mercury salts.

A. When fertilized *Echinus* eggs were placed in KCN *n*/50,000[1] growth ceased at the 2- or 4-cell stage. A small percentage of eggs were cytolysed and many had multiple asters and irregular segmentation. In KCN *n*/100,000, after 24 hours more eggs were cytolysed, a certain proportion had got no further than the 2- or 4-cell stage, and a small percentage had become blastulæ, most of them of the abnormal solid type (stereoblastulæ). The controls had in the same period reached the gastrula stage.

After 72 hours, 24 hours after the controls had reached the early pluteus stage, these blastulæ had become late gastrulæ. These were transferred to sea-water, and developed as far as the pre-pluteus stage, but never became normal plutei, thus bearing out Child's conclusions that considerable poisoning in the early stages in some way disturbs the relations of parts so that even when the developing organism is replaced in normal conditions, it can only develop up to a certain stage, and no further.

In this connection the observations of Perkins ('02) are of interest. He discovered that the hydriform larva of *Gonionemus* when kept in the laboratory lost its typical form and assumed an irregular amœboid shape. It moved about, apparently ingested food, repeatedly underwent a form of fission, and lived for over 2 months.

Obviously, therefore, viability and capacity to develop are by no means synonymous, and we have the theoretical possibility of the existence of persistent larval forms due to unfavorable conditions as well as to genetic variations.

Other larvæ were replaced from KCN *n*/100,000 to sea-water at 48 hours, from the blastula stage. After a further 48 hours, many had formed early plutei. These were mostly of a very wide-angled type (Fig. 9), thus showing what Child finds in similar circumstances, and has called "differential recovery."

Thus between the 48th and the 72nd hour in the solution the larvæ had lost their power of recovery in sea-water, though not of continued existence.

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[1] The molecular concentration is simply given for convenience: the actual ionic concentration of CN would naturally vary with the hydrogen-ion concentration and other factors of the sea-water used.

B. $HgCl_2$. $n/5{,}000$, $n/10{,}000$, $n/20{,}000$, $n/50{,}000$ and $n/100{,}000$ solutions allowed segmentation to proceed to the 2-, 4-, or 8-cell stage in 24 hours (mostly to the 4-cell stage). Many segmenting eggs, however, were obviously damaged, and by 48 hours all were dead. The course of events was similar in a $n/200{,}000$ solution, except that by 24 hours segmentation had proceeded to the 4- to 32-cell stage (mostly to the 8-cell stage). Transference from $n/100{,}000$ and $n/200{,}000$ to sea-water at 24 hours had no effect, all being dead at 48 hours. The dead blastomeres were well-preserved, not cytolysed or disintegrated.

As the solutions used were obviously too strong, 24-hour gastrulæ from the control were placed in $n/500{,}000$ and $n/1{,}000{,}000$ solutions. These too were all dead 24 hours later (48 hours from fertilization); but they showed the interesting phenomenon of differential disintegration. All the tissues except the archenteron had entirely disintegrated, and lay as a sheet of cell-débris on the bottom of the vessel. The archenteron, on the other hand, was well preserved, and the outlines of its walls could be clearly seen (Fig. 10). The spicules were usually to be seen adherent to it. Sometimes it appeared solid. This is obviously an occurrence of the nature of those observed by Child ('15), and utilized by him in his axial gradient theory.

3. Dedifferentiation of Larvæ.

Unfortunately no further *Echinus* were to be had, and I was therefore unable to repeat the experiments with more suitable strengths of solution. Wishing, however, to see what the effect of poisons might be on more advanced stages, I transferred some of the plutei from the controls to various solutions of KCN and $HgCl_2$. The most interesting results were obtained in the mercuric-chloride solutions.

C. A preliminary experiment was made with 2-day plutei in $HgCl_2$ $n/1{,}250{,}000$. After 6 hours, many showed a retraction of the arms, leaving part of the skeletal spicules protruding. After 24 hours, many had died. The following types could be distinguished:

1. Partially dead, with disintegration of the tissue of the arms; the aboral ectoderm not disintegrated, or with a few cells migrated

out of the tissue and adherent to it externally (these cells appeared normal, in contradistinction to the pathological granular disintegration of the tissue of the arms). The form of the gut was well preserved, and no disintegration of any sort was visible in it (*cf.* Section 2, *B*).

In some cases it appeared that all tissues had disintegrated with the exception of the somewhat contracted gut, which was thus left by itself; but I was unable to be quite sure of this.

2. Slightly shrunken plutei with the skeleton protruding through the arms; these latter might be from 25 to 75 per cent. of their original length.

3. As (2) but the skeleton not protruding from the arms. The terminal portions of the spicules had apparently been resorbed by the arms as they contracted.

All the plutei had sunk to the bottom, and ciliary action was so much reduced that only very sluggish movement was taking place.

In addition to the types mentioned, others were seen which were of spheroidal form, dense, without arms, and with simple or no spicules. I at first thought that these might be examples of extreme dedifferentiation, but on examining the control culture, I found at the bottom a certain proportion of quite similar organisms. The culture was slightly overcrowded and these were doubtless susceptible individuals inhibited by the slightly adverse conditions. These types are mentioned to draw attention to the necessity for careful control in similar experiments. (This has already been emphasized by Shearer, De Morgan and Fuchs ('13), p. 274, etc.) They are also interesting as indicating that considerable inherited variations in vigor and resistance occur among the offspring of a single pair of *Echinus*.

In the later experiments now to be recorded, the plutei were picked out from the control culture with a fine pipette under the binocular microscope, transferred to the medium to be tested and there examined, to see if any of these minute armless forms had been transferred by mistake. In no experimental vessel was more than one of these forms discovered immediately after transfer; it can therefore be safely assumed that in this series the experimental animals were for practical purposes all healthy plutei with well-developed arms.

D. Four-day plutei transferred to $n/3,000,000$ and $n/4,000,000$ $HgCl_2$. After 24 hours, all appeared normal, and about 90 per cent. were still swimming. After 48 hours, all had sunk to the bottom, ciliary action was somewhat reduced, there was a slight general shrinkage and loss of transparency as compared with the controls, and the arms had become slightly though definitely shorter, but without protrusion of the skeleton. The experiment was here discontinued, after another series had been started.

E. Five-day plutei (*a*) to $HgCl_2$ $n/1,000,000$, (*b*) to $HgCl_2$ $n/2,000,000$, (*c*) control in same amount of outside sea-water.

The controls remained healthy, with long arms, throughout the first 4 days of the experiment. The results may be tabulated as follows:

Table I.

	(*a*) $\frac{n}{1,000,000}$.	(*b*) $\frac{n}{2,000,000}$.
24 hrs.	All at bottom. Marked arm-resorption; no protrusion of spicules; arms mere knobs or ¼ to ½ normal length; aboral end usually swollen.	All at bottom. Moderate arm-resorption; no protrusion of spicules.
48 hrs.	Still further arm-resorption.	Slightly more resorption than at 24 hours.
72 hrs.	Arms absent or only knobs; a few with total dedifferentiation of oral end; many with aboral end no longer swollen, mesenchyme clumped, gut shrunken, spicules sometimes just protruding.	Slightly more resorption than at 48 hours.
96 hrs.	Spicules often protruding, most showing disintegration at oral end, and narrow form of body.	Condition as in (*a*) after 24 hours.
120 hrs.	Dead.	Condition as in (*a*) after 48 hours.
144 hrs.		Condition as in (*a*) after 96 hours.
168 hrs.		Dead.

(*a*) $n/1,000,000$. Some individuals after 24 hours in the medium are represented in Fig. 1.

The controls had arms about 25 per cent. longer than Fig. 1, *c*. It will be seen that in all cases the arms have diminished in length. They have also become more dense in appearance, being in the more advanced examples quite opaque. The diminution is rarely less than that seen in Fig. 1, *c*, and ranges from about 20 per cent. to about 80 per cent. The skeleton does not protrude at the tips of the arms, and the missing parts have apparently been resorbed. Another marked feature in the large majority of the specimens is the dilatation of the trunk and aboral regions (Fig. 1, *a–c*), which leads to a bulgy form, usually with a wide separation of the aboral ends of the spicules.

This may be explained as an effect of differential susceptibility, the more susceptible oral region contracting before the aboral, which then becomes distended with fluid. In some specimens (Fig. 1, *c*) the trunk ectoderm was seen to be definitely thinner than normal, implying distention. Other specimens (Fig. 1, *d* and *e*) did not show these phenomena at all markedly; in every case, such non-distended individuals exhibited extreme arm-resorption, and were also well below the average size. These were presumably individuals of general low susceptibility, in which all regions had suffered, the aboral ectoderm also being contracted, and the total volume of body-fluid being in some manner diminished. In point of fact, they were usually seen (Fig. 1, *d*) to have abnormally thick aboral and trunk ectoderm.

The number of pigment-granules appeared in general to have increased.

The appearances after 48 hours were very similar, with an increase in the amount of arm-resorption, and of aboral contraction.

After 72 hours, none had more than quite vestigial arms (Fig. 2, *a*), while the majority were almost or quite armless (Fig. 2, *c*). Very few now showed the separation of the aboral ends of the spicules, and not so many the swelling of the trunk region (Fig. 2, *c*). Many, on the contrary, were very narrow, especially at the aboral pole, and showed a condensation of the mesenchyme cells to form clumps (Fig. 2, *a*). In Fig. 2, *b*, the aboral region of the same specimen is shown under a higher power. The large clumps of mesenchyme cells are extremely like the clumps of blood (mesenchyme) cells seen in dedifferentiated specimens of the Ascidian *Clavellina* (*cf.* Driesch, '06).

Up till 48 hours, the gut had remained apparently normal. In these specimens, however, though the stomach was of normal (Fig. 2, *a*) or even more than normal size (Fig. 2, *c*) the œsophagus and intestine showed some degree of contraction.

Extreme cases of dedifferentiation at 72 hours are shown in Figs. 2, *d* and *e*. In Fig. 2, *d,* the general turgescence or tone, and the transparent appearance of the aboral region is preserved, but the oral region has rounded off, and all traces of the characteristic form of the oral half of the animal, including the ciliated band, have disappeared. The stomach too is affected, all parts of the gut being now reduced, with thick epithelium.

Finally in Fig. 2, *e,* we have a specimen with complete dedifferentiation of the oral region. The aboral region, however, is also markedly affected, and the whole body is filled with a nearly opaque mass of cells, within which only faint traces of organs are visible. The skeleton is reduced to two simple clubbed rods. In this condition the animal much resembles a dedifferentiated *Clavellina,* and the resemblance would externally be almost complete if it were not for the presence of the spicules, which prevent the retraction of the aboral region and the assumption of the spheroidal form.

At 96 hours, most of the specimens showed signs of partial death. This manifested itself in the oral region by a disintegration of some of the tissue, and the protrusion of the skeleton. The gut was in all cases contracted. The aboral region was usually contracted, but with no trace of disintegration. (Fig. 3, *a* and *b*.) Most of them were transferred to sea-water. Those that remained in the solution were all dead after 120 hours, with aboral as well as oral disintegration, but very little or no disintegration of the gut. Slight movement due to ciliary action, and occasional contractions of the œsophagus were seen up to 96 hours.

One interesting occurrence was noted after 72 hours. Two plutei had grown together, the left anal arm of one having completely fused with the aboral region of the other, the skeletons of the two individuals overlapping in the common region (Fig.

4). The pair was isolated in sea-water, but was dead by 120 hours.

The progress of dedifferentiation in $HgCl_2$ $n/2,000,000$ was similar but considerably slower.

This is shown in Fig. 5, which represents an advanced specimen after 120 hours (by which time all those in $n/1,000,000$ were dead). An abnormal addition to the skeleton is seen. This may perhaps be due to the deposition in the aboral half of calcium carbonate resorbed in the arms, or to the fact of the spicule-secreting tissue being less affected than the rest, as Child ('16) suggests. The mouth was, I think, closed; the œsophagus was swollen. Most individuals after 120 hours were in the same stage of dedifferentiation as shown by the $n/1,000,000$ culture after 48 hours.

Partial death was not seen till 144 hours; complete death had occurred by 168 hours. As in the $n/1,000,000$ culture, the still-living portion when partial death had set in was very narrow, the spicules being nearly parallel. This narrowness is a result of all regions being affected simultaneously.

The controls for this experiment remained very healthy and off the bottom for 96 hours. The culture was rather crowded and not fed. After 120 hours, most were on the bottom, and were showing a certain amount of arm-resorption (though less than that shown in $n/1,000,000$ after 24 hours). One was seen with 3 arms reduced to minute knobs, but the fourth perfect. This was never seen in the $HgCl_2$ cultures, although there the arms of one side might be resorbed faster than those of the other.

The resorption in the controls might have been due either to starvation, or to the accumulation of toxic products, or both, but tests were not made to determine the point. Runnström's results ('17), show that either alone can cause dedifferentiation.

All were on the bottom after 144 hours. They remained in this position with almost total absence of arms, but healthy in other respects, for 5–7 days longer.

F. Nine-day plutei from the main control culture (fed on *Nitzchia*) were transferred to $HgCl_2$ $n/1,000,000$.

The appearance of the more advanced of these is shown in Fig. 6. All had developed the third pair of arms.

After 24 hours all had become somewhat reduced in size, with slightly denser appearance. The gut too was shrunken. The arms, especially the third pair, usually showed slight reduction in size.

After 48 hours, marked changes had occurred (Fig. 7). The third pair of arms had in all cases totally disappeared, and the others showed considerable but variable resorption. The previously noted swelling of the trunk and aboral region was present to a greater or less degree. The most interesting change was seen in the gut, which was always contracted, and contained a greater or less number of small round bodies. On examination with a higher power, these proved to be cells, practically spherical, and not cohering. They had presumably migrated out of the stomach epithelium into the lumen.

This is paralleled by the migration of the cells out of the aboral ectoderm in Expt. *C* above (pp. 212–213), and by the behavior of the tissues in organisms that dedifferentiate by resorption, as in *Perophora* (Huxley, '21 *b*) and Hydroids. (Loeb, '00. Huxley and de Beer, in press.)

After 72 hours, dedifferentiation had progressed much further (Figs. 8, *a* and *b*). The size is much less, the arms very small and extremely dense, the gut quite packed with cells, and much contracted. The contraction of the gut has expelled some of the cells at the anus and sometimes also at the mouth. The trunk and aboral regions are sometimes swollen, more usually contracted. A fair number of plutei had died.

At this stage the survivors were replaced in sea-water.

KCN. Experiments on 4-day plutei in KCN *n*/100,000 and *n*/200,000 gave on the whole similar results to those in $HgCl_2$ *n*/1,000,000. Disintegration of the trunk ectoderm was never observed, and is possibly a specific effect of Hg (*cf.* Child, '17). Arm-resorption was not quite so rapid.

4. Experiments on Recovery.

These are very incomplete, owing to lack of material. Through the courtesy of Mr. J. Gray, who independently observed dedifferentiation phenomena, in larvæ treated with citrates, I am enabled to state that in this case some degree of recovery at least is possible. It would here appear that forms which have resorbed their arms (similar to my Fig. 1, *b*, or 1, *d*) are able to regenerate them, while those which have dedifferentiated to a completely spheroidal mass can probably redifferentiate to a pre-pluteus (helmet-shaped) stage at least. Mr. Gray, however, proposes to investigate the matter further, and I merely quote his preliminary results in order to show that dedifferentiation does not preclude recovery, at any rate to some degree. As is well known, dedifferentiation in *Clavellina* and in *Protozoa* does not preclude complete recovery (Driesch, '06; Lund, '17, etc.).

The observations I have made, however, on forms dedifferentiated by KCN and $HgCl_2$ (in which case dedifferentiation appears to be slower than when caused by citrates) have not yet shown recovery, though many specimens lived for weeks in sea-water, and ingested food. From the present results, it would seem as if the poison had robbed the organism of its capacity for development, but not of its capacity for maintaining life. I must emphasize that owing to lack of more ripe *Echini*, I was unable to do more than carry out preliminary observations, and that systematic experiments on the subject are in view.

That being so, I will record my results in the briefest possible manner. Most moderately-dedifferentiated forms (*i.e.*, with ciliated band, but arms absent or vestigial) transferred to sea-water from KCN remained alive and in approximately the same condition for over 4 weeks. They kept on the bottom, and retained a slight motility. In one culture the beginnings of recovery were noted in the shape of increased motility and of a few larvæ beginning to swim freely once more, but after a few days they again reverted to their former condition. In all cultures a slight *progressive dedifferentiation* was noted after 1 to 2 weeks, and towards the end of the 4 weeks, a larger proportion of spheroidal forms was found.

On adding *Nitzchia* to two cultures, it was found that diatoms were ingested by many of the dedifferentiated larvæ. These cultures lived better than those without *Nitzchia.*

A number of these larvæ were also placed, after 2 weeks, in a jar with *Nitzchia* and a stirring apparatus; but 3 weeks later none could be discovered.

The history of those transferred to sea-water from $HgCl_2$ is similar in essentials. The larvæ lived up to 3½ weeks. Some ingested *Nitzchia* when this was provided. Progressive dedifferentiation was, I think, more pronounced in these $HgCl_2$-treated larvæ.

Figures are given of some of the types seen during this progressive dedifferentiation. Fig. 11 shows a larva which has completely lost its arms and also its ciliated band, together with the antero-lateral and transverse skeleton on one side. The remainder of the skeleton permits it to retain some of its characteristic form. The gut and its epithelium are contracted, and its spatial relations altered. The body-cavity is clear, with a few clumps, some of pale cells, others of red pigment-cells. The general appearance is much clearer and less full of cell clumps than in larvæ in advanced stages of dedifferentiation still in the toxic solution.

Fig. 12 shows a further stage of loss of form. Here the outline is simply spheroidal. The broad œsophagus, contracted stomach and thin intestine lie approximately in a straight line. The body-cavity is very clear, with the exception of a few large clumps of cells. The aboral clubs have been broken off from the rest of the skeleton. This breaking of the skeleton, it should be noted, was frequently seen in forms where the aboral and trunk regions were much dilated. The change of form of the aboral region exerts a pressure inwards on the rods, at right angles to their axis, and snaps the ends off; at first they lie in the position in which they have been broken, thus making it easy to see how breakage occurred. Once this happens, the oral ends of the rods will no longer be pressed against the body wall, and the whole skeleton thus ceases to function as a support. This is clearly seen in Fig. 12, *a* and *b*.

The general appearance of the tissues in this larva, and indeed in most of those in the recovery experiments, is perfectly healthy. Even after the skeleton has disappeared and the spheroidal form has been assumed, traces of the "lip" or pre-oral lobe may often be seen near the mouth. This also, however, has disappeared in the larvæ shown in Fig. 12.

A very advanced stage of dedifferentiation is seen in Fig. 13 *c*. The larva had shrunk considerably in size. This is apparently due to the contraction of the ectoderm, which was cuboidal instead of flattened. No trace of skeleton was present. (The animal was examined both *in vivo* and as a stained preparation.) Large, brownish aggregations were seen in the interior, together with an apparently closed and solid pale vesicle, perhaps the stomach. Curious irregularities of the ectoderm were observed at one pole. These were also seen in several other specimens (*cf.* Fig. 12 *b*).

The general resemblance of this specimen to much-dedifferentiated individuals of the Ascidian *Clavellina* is striking (Driesch, '06; Schultz, '07; and my own unpublished observations). There are numerous points of difference, as one would expect in such widely different organisms, but the following essential similarities exist: (1) the assumption of the spheroidal form; (2) the aggregation of free cells to form dark masses; (3) the regression of epithelial cells to the cuboidal condition; (4) the conversion of internal structures into closed vesicles; (5) the congested condition of the body spaces, consequent upon contraction.

5. On the General Effect of Mercury in Dilute Solutions.

In order to get some more accurate idea of the processes occurring in a weak solution of a mercury salt, some experiments were carried out on the gill of *Mytilus*. I have to thank Mr. J. Gray, of King's College, Cambridge, for some suggestions.

A preliminary test with various strengths of $HgCl_2$ solution showed that in very weak solutions marked disintegration of the tissue took place before ciliary action was stopped.

The point to be tested was whether the effect of Hg^+ ions was proportional to the *strength of solution* used, or was a progressive effect, proportional to the *total amount* of mercury in the solution.

(*A*) Five finger bowls were prepared, and 4 pieces of gill placed in each. One contained 50 c.c. of sea-water as control; the others 50 c.c. of $n/375{,}000$ $HgCl_2$. The solution in Nos. 1 and 2 was not changed. In No. 3, it was changed after 2, 4, 6, 8 hours and again after 24 hours. In No. 4, it was changed every ½ hour for 8 hours, and again after 24 hours.

After 3 hours, there was slightly more disintegration in No. 4 than elsewhere. After 6 hours, all the pieces in No. 4 had their terminal rounded portion markedly disintegrated, and the current produced by the lateral cilia had ceased.

In Nos. 1 and 2, one piece in each was similar, but the others had scarcely begun to disintegrate. No. 3 was intermediate. In Nos. 1, 2, and 3 the current produced by the laterals was still evident.

After 24 hours, No. 4 was very markedly disintegrated, and no ciliary movement was visible. In Nos. 1 and 2, 4 of the 8 pieces showed ciliary movement, and the disintegration was not so marked as in No. 4. No. 3 was intermediate. The conclusion was apparently to be drawn that it is the total amount and not the concentration of the Hg$^+$ ions present that is operative; but the concentration used was apparently too high. The experiment was therefore repeated in a modified form: (*B*) Five finger bowls were prepared with 5 pieces of gill in each. In them were placed respectively 5, 20, 50, 100 and 300 c.c. of a $n/1{,}500{,}000$ HgCl$_2$ solution.

The following table gives the results observed (+ = strong ciliary action; ⊕ = moderate; ⊕ ⊕ = faint; ⊕ ○ = very faint; ○ = no ciliary action. ((D)) = slight disintegration; (D) = medium; D = marked disintegration.

TABLE II.

No.	c.c.	Time in Hours.					
		3	21	27	45	70	
1.....	5	+	+	+	⊕	⊕	All proximal cut ends healed.
2.....	20	+	+	+	⊕	⊕ ((D))	
3.....	50	+	⊕ ((D))	⊕ (D)	⊕⊕ (D)	......[1]	All proximal ends unhealed; dead after 45–70 hrs.
4.....	100	+	⊕ ((D))	⊕⊕ D	⊕⊕ D	......[1]	
5.....	300	+	⊕ ((D))	⊕⊕ D	⊕⊕ D	⊕○ D	

It will be seen that the effect of the HgCl$_2$ was a function of the total amount used, all the solutions being of the same strength. The death of the proximal portions of the filaments was very striking in the large bulks of solution.

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[1] Numbers 3 and 4 were not observed at 70 hours, owing to an oversight.

There was, however, very little difference between the effect of Nos. 4 and 5. That is to say, the maximum effect possible with a solution as weak as *n*/1,500,000 is, with the amount of tissue used in the experiment, attained with a total bulk of about 100 c.c.

In any experiments concerning the effects of salts of the heavy metals upon living cells, it will therefore be necessary in every case to consider not only the strength but also the amount of the solution, and further the amount of living tissue on which experiments are being carried out. Failing this, the results obtained will not be comparable. However, since in the experiments on plutei here recorded, the bulk of $HgCl_2$ solution was large in comparison to the bulk of the plutei, and since the results are only qualitative, they are not invalidated by the conclusions just reached.

6. Discussion.

A. *Maintenance of Form.*

Recent work is coming to show more and more clearly how organic form is the product of an equilibrium between constitution (internal forces) and environment. This applies equally well to whole organisms or to their parts. In order that the typical form may be maintained, a particular complex of environmental stimuli is necessary.

In the case of Ascidians such as *Clavellina* (Driesch, '06; Schultz, '07) and *Perophora* (Huxley, '21 *b*) whole zoöids below a certain size when exposed to unfavorable agencies, such as accumulated waste products or dilute solutions of KCN, are unable to maintain their form, and undergo what is known as dedifferentiation.

The same is true of Hydroid polyps (Loeb, '00). In Hydroid polyps we (Huxley & De Beer, in the press) have succeeded in finding a quantitative relation between the rapidity of the form-changes and the concentration of the toxic solutions employed; this is also seen in the present study (Table I.).

The encystment of *Protozoa* in times of drought or cold is an example of the same phenomenon.

The remarkable dedifferentiation of the hydriform larva of

Gonionemus observed by Perkins ('02) should again be mentioned here.

As regards parts of organisms, attention may be called to the phenomena seen in sponges (Minchin, '00, p. 29). When an Ascon type of sponge contracts, the internal environment is altered, and the collar cells are unable to maintain their typical form, losing their collar and flagellum and becoming spheroidal. See also Huxley ('21 *a*, p. 313).

The familiar resorption of many grafted tissues may also be mentioned. Muscle fibers, like *Clavellina,* also dedifferentiate preparatory to regeneration when cut across. (Towle ('01).)

Even the form of mental organization is subject to the same limitation. In certain "shell-shock" and other cases, strain and unfavorable environment render the higher part of the mental organization unable to maintain itself, resulting in what is known as *regression*. See Nichol ('20).

We may thus say that maintenance of normal form is possible only in certain environments. Certain stimuli result in what may be called hyper-typical form: *e.g.,* in regenerating Planarians, high temperature produces forms with exaggerated heads (Lillie and Knowlton, '97; Child, '15, p. 138). On the other hand, many unfavorable stimuli do not permit the establishment of the type at all: they result in infra-typical form—*e.g.,* cold in regenerating Planarians; below a certain temperature, no head is formed (*auctt: cit.*).

B. *Dedifferentiation and Metamorphosis.*

The resemblance of the phenomena here described to those occurring at the metamorphosis of the pluteus is very striking. According to MacBride ('02) the course of the process is as follows: The larva sinks to the bottom, presumably as a direct result of the weight of the growing *Echinus* rudiment. The arms are next resorbed, those on the same side as the *Echinus* rudiment first. The larval œsophagus contracts. The ectoderm of the ciliated epaulettes is "invaginated" and devoured by amœbocytes (the description, however, does not negative the possibility that the cells of the epaulettes may migrate out of the tissues, rather than amœbocytes migrate in). During arm-resorption, the ectoderm of the arms shrinks, leaving the spines exposed "exactly as in unhealthy larvæ at all stages of development." The oral lobe and the outer part of the œsophagus disappear. The inner part of the œsophagus persists for a time as a completely closed tube. Very remarkable changes occur in the stomach. In the late larva it is highly turgid, with cells intermediate between cubical and flattened. It now loses its turgidity; the walls become very thick, and eventually folded, the lumen often almost disappearing. The cells are stated to multiply with great rapidity, but definite proof of this is not given, and quite possibly the appearance of increased number is due to the contraction of the wall; this point deserves re-investigation. The cells round themselves off, and many migrate into the surrounding jelly. The new stomach is reconstituted from the residue.

While this has been occurring, all resemblance to an Echinopluteus form has disappeared, and the animal becomes almost hemispherical.

The essential points to be noticed are as follows:

(1) The resorption of the arms. This appears to be identical with what we have seen in larvæ placed in toxic solutions.
(2) The dedifferentiation of the specialized larval organs, the epaulettes. These are not present in the earlier larvæ used by me.
(3) The loss of the general form of the larval part of the organism, and its approximation to the segment of a spheroid.
(4) The contraction of the œsophageal tissue.
(5) The closure of the mouth and formation of a closed vesicle from the remains of the œsophagus.
(6) The contraction (loss of turgidity) of the stomach.
(7) The migration of some of the cells of the stomach out of the tissues. This was paralleled in my experiments, though there the cells migrated inwards to the lumen, instead of outwards to the body-cavity.

As far as the destruction of larval organs goes, we can assert that the dedifferentiation caused by toxic solutions and the reduction at metamorphosis are closely and essentially similar. The difference between the end-results is presumably due to the fact that at metamorphosis there is present a new organic system, the developing *Echinus* rudiment, which was absent in the subjects of the dedifferentiation experiments. We may suppose that the metamorphic changes in Echinoids are normally initiated somewhat as follows: the inherited constitution of the animal leads to the production of the *Echinus* rudiment. The weight of this leads directly to the sinking of the larva to the bottom. The subsequent changes would then be due to two causes: (1) The conditions at the bottom are directly unfavorable to the larval organs, which therefore are unable to maintain themselves, and so start dedifferentiating. (2) The developing adult organs are not inhibited by the benthic environment, and their continued growth and consequent demands for nutrition accelerate the dissolution of the larval system.

The stomach is an organ which is remodelled. It is unable to maintain itself in its typical larval form, and regular dedifferentiation-changes start in it. But the activities of the adult organs provide a new internal environment, and the remains of its tissues, entering into equilibrium with this, form the rudiment of the adult stomach.

It should follow from these considerations that precocious metamorphosis should be induced by placing larvæ with a developing *Echinus* rudiment in dilute toxic solutions. This conclusion it is intended to test by experiment. Meanwhile Professor MacBride informs me in conversation that those of his cultures in which conditions are not optimum, do as a matter of fact exhibit precocious metamorphosis. In such conditions, the larvæ sink to the bottom while the *Echinus* rudiment is still in a stage much less advanced than that which it possesses at metamorphosis in the best cultures. In spite of this, metamorphosis takes place, but leads to the production of small, weakly, under-developed Echini. I am grateful to Professor MacBride for informing me of this confirmation of my theoretical considerations.

Loeb ('00) made the suggestion that the retrogressive changes of histolysis in metamorphosis were comparable to dedifferentiation as seen in Hydroids, but I am not aware that the similarity between dedifferentiational and metamorphic changes in one and the same species has yet been pointed out, as here in *Echinus*.

If these considerations prove to hold good, it will follow that phagocytosis is a secondary phenomenon in the metamorphosis of Echinoids, and probably of other groups. It will, however, obviously be our next task to test by experiment the hypothesis that the dedifferentiation of larval tissues is the essential factor initiating Echinoid metamorphosis.

C. *Axial Gradients and Surface-effects.*

Child's theory of axial gradients and the differential susceptibility along them has been set out at length in his books ('15, etc.) and papers, and it is unnecessary to enter into it here.

I would like to point out, however, some facts which may lead to a modification of some minor parts of the theory.

Child measures and delimits his "metabolic gradients" by means of the differential susceptibility of different organs to toxic agencies. In certain cases this gives concordant and uniform results. In other cases the susceptibility of an organ is found to vary during development in a way which is not to be explained without demanding a very considerable elasticity from the theory.

The general bases of the theory appear to be founded on solid enough foundations, the main difficulty being that they are perhaps too general, the term "metabolic rate," for instance, being only capable of application in an unanalysed sense, as an expression of general total activity. But numerous exceptions, such as the unpredictable variation in susceptibility of organs above alluded to, can I think be explained by reference to another and simpler notion. That is, that cells are more susceptible and more prone to dedifferentiation in proportion to the amount of surface which they are exposing. We can put this in another way, and say that a cell maintains its form with greater difficulty when its surface is large than when it is small.

This statement is based on numerous facts, including the following observed by myself, as well as being deducible from theoretical considerations.

(1) In the Ascidian *Clavellina,* the parts first showing dedifferentiation, and finally most dedifferentiated, are those where the normal cells expose a great deal of surface (pharynx and atrium).

(2) The same is true of the related form *Perophora,* in spite of its different mode of dedifferentiation.

(3) In *Perophora,* the stolon-ectoderm is usually flat. When treated with toxic agents of a certain strength, it becomes cuboidal.

(4) In sponges, the collar-cells exposed to very mildly toxic agents retract their collars. Further toxicity causes the assumption of the spheroidal form by the cell. (Huxley, '21*a*.)

(5) In dissociation experiments, the shock of mechanical separation causes the assumption of the spheroidal form, and the loss of any differentiated structures such as collars, flagella, or pseudopodia, in all types of cell in sponges. (This is true also in other sponges (H. V. Wilson, '07) and in Cœlenterates (H. V. Wilson, '11; De Morgan and Drew, '14).)

(6) In the plutei here described, the gut is at first extremely resistant to toxic agents. Later, however, it becomes very susceptible to them. This alteration in susceptibility is accompanied by an alteration in appearance, the gut passing from a thick-walled organ to an extremely turgid, thin-walled one. This turgidity has also been noted by MacBride ('02); the cells in this latter condition are very much flattened, with a relatively enormous surface. One of the first effects of $HgCl_2$ solutions in later larvæ is to cause a shrinkage of the gut and a separation and rounding-up of some of its cells.

It is of course presumable that high energy-consumption is necessary to maintain a cell in a flattened condition, or in any other involving a large surface area—as would indeed be expected from the laws of surface-tension. But to say that a high "metabolic rate" thus produced is to be reckoned in the same category as the high metabolic rate of a "dominant" region as defined by Child, is to reduce the value of the whole very important conception of physiological dominance.

Further, in cells grown in vitro, Holmes ('14) notes as a general rule that any unfavorable condition leads to the abandonment of an extended for a spheroidal shape. Numerous other instances could be cited, but the phenomenon is so widespread as to be familiar to all.

We may therefore say that, apart altogether from the question of axial gradients, the susceptibility of cells to toxic agents, and their readiness to dedifferentiate, are in part functions of their surface area.

D. *Previous References to Dedifferentiation in Echinoid Larvæ.*

Vernon ('94) found that various environmental factors had a marked effect on the arm-length of plutei (the experiments were continued up to the 8-day stage). The effect, whether caused by actual resorption of tissue present, or failure to grow beyond a certain length, means that equilibrium with these slightly unfavorable conditions could only be maintained by arms of a shorter length than normal.

Robertson ('13) found that *Strongylocentrotus* blastulæ transferred to a medium containing 0.15 per cent. lecithin for 24 hours and then returned to sea-water, not only were very much retarded in development, but after becoming gastrulæ nearly lost their gut again before dying (figures and details are not given). He ascribes this result to a specific action of lecithin on growth-processes. From what we know of Echinoderm development, the result, in the absence of further data, might at least equally well be non-specific and due to dedifferentiation.

In addition, the literature abounds with notices that "unhealthy" larvæ may show shortened arms, sometimes with a terminal protrusion of spicules. (And see postscript, p. 230.)

E. *Recovery.*

The meager results on recovery merit one or two words. Future experiment must decide between one or two possibilities. Either the Hg^+ ion damages only a fraction of the cells' protoplasm, which, if it does not exceed a certain critical value, may be repaired, and normal differentiation resumed; or repair is not possible, and the animal cannot differentiate further. The theoretical bearings of this second possibility have been discussed by Child ('16) and I do not intend to go into them at present. What is interesting in either eventuality is the extremely long period for which the dedifferentiated organisms can remain not merely alive, but, it should be emphasized, with apparently quite healthy tissues.

The fact that such larvæ ingested diatoms in a normal fashion is noteworthy. The possibility is thus raised of producing, by environmental changes, permanent larval forms. (*Cf.* Perkins, '02.) Such permanent larval forms of small size, but hereditarily determined, are of course known in many species, especially in the male sex.

The action of mercury is apparently to precipitate and put out of action a definite quantity of the living molecules in the cell.

POSTSCRIPT.

Owing to delay in the arrival of German periodicals after the war, I did not see the important paper by Runnström ('17) until I had not only completed the work here recorded, but also written the paper. Runnström has demonstrated that young Echinoid plutei treated with $ZnSo_4$ in very weak solutions undergo dedifferentiation-changes very similar to those observed by me. He also finds that dedifferentiation occurs spontaneously in a certain proportion of larvæ in every culture, especially from over-ripe eggs. The specimens noted by me on p. 213 above are doubtless of this category. Finally, he describes very similar dedifferentiation as the result of starvation, from which recovery is possible. Recovery is also possible in the $ZnSo_4$ larvæ when replaced in sea-water.

I will not summarize his results further, except to say that they show conclusively that dedifferentiation may be produced by many agencies, and may be reversible.

There are one or two points of general theoretical interest which may be noted. While remarking on the resemblance of the changes seen at metamorphosis to those produced by starvation, Runnström says that an *essential* difference between the two is the rapidity of the metamorphic changes. On the other hand, reduction by means of toxic agencies is more rapid, and it should be remembered that at metamorphosis the existence of rapidly growing imaginal organs will drain the rest of the organism very quickly. The rapidity of dedifferentiation in the zoöid of *Perophora* is very great—*once it has started.* The observations of Professor MacBride (p. 226) also corroborate strongly the idea that toxic agencies are important in the initiation of metamorphosis. Cytolysing agencies are not necessary to explain resorptive dedifferentiation such as occurs in Hydroids and *Perophora;* they should therefore not be postulated, as is done tentatively by Runnström, to explain metamorphic changes in Echinoderms until definite proof can be adduced of their existence.

A further corroboration of the idea of metamorphosis as an upset of balance is to be seen in Runnström's own remarkable observations upon the formation of pedicellariæ. In much-reduced larvæ, more or less well-differentiated rudiments of pedicellariæ often appear *ab initio.* Once pedicellariæ have been formed, however, they are among the first organs to be dedifferentiated. This may be interpreted by supposing that the potency of producing pedicellariæ is present in larvæ, but normally inhibited until a certain stage by the demands of the existing organs. When these are reduced, however, the check is removed and the pedicellaria develops. Once developed, on the other hand, its cells are not actively growing, and are easily affected by unfavorable agencies. This is very similar, it would seem, to the behavior of larval and adult organs at metamorphosis.

With Runnström's general discussion I find myself in full agreement in all essentials, except that I do not consider that he has laid sufficient stress upon the direct inhibiting effect of poisons upon growing tissues, an effect which leads eventually to dedifferentiation by a different route from that consequent upon hunger.

April 6, 1921.

Summary.

1. The retardation of early *Echinus* development caused by toxic agents (KCN and $HgCl_2$) is noted.

2. When recovery took place on transference to sea-water after 48 hours in the toxic solutions (*i.e.,* in the blastula stage), plutei were formed. These were mostly very wide-angled; this was due to the greater power of recovery of the oral region.

3. On transference to sea-water after 72 hours' treatment with the poisons (*i.e.,* in the gastrula stage), development only took place as far as the pre-pluteus stage. Considerable power of re-

covery is thus lost by prolonging the treatment with poisons from 48 to 72 hours.

4. Gastrulæ killed by weak toxic solutions show *differential death,* the enteron being more resistant than the rest of the tissues.

5. In very weak solutions, progressive *dedifferentiation of plutei* occurs. The arms are first resorbed, then the ciliated band and oral lobe disappear, then the gut contracts. In early stages, the trunk and aboral end are dilated, in later stages they are contracted and the whole body is filled with cells. The process is similar in all essentials to that seen in the dedifferentiation of the Ascidian *Clavellina.*

6. It could not be decided whether full *recovery* is possible for plutei thus treated. Armless forms replaced in sea-water remained alive and motile for 3½ to 4 weeks, and ingested diatoms. Some of them showed *further dedifferentiation* in the sea-water, finally reaching a spheroidal state, with spicules extremely reduced or absent, and gut reduced to a closed vesicle or vesicles.

7. The process of dedifferentiation appears to be essentially identical with that initiating *metamorphosis* and resulting in the destruction of the larval organs in Echinoids. This is probably true also for metamorphosis in other groups.

8. Echinoid metamorphosis, and possibly other types of metamorphosis also, would therefore appear to be *initiated* by dedifferentiation-changes in the larval organs, not by autolysis or phagocytosis, which are secondary phenomena.

9. Ceteris paribus, tissues with *large cell-surface* are more susceptible to unfavorable influences and more prone to dedifferentiation than are those with a small area of surface per cell.

10. The effect of mercury salts in dilute solutions is shown to depend upon the *total amount* present as well as upon the *concentration.*

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LIST OF FIGURES.

All figures were drawn from life with the aid of an Abbé camera lucida, at table level. All are drawn with the combination of a Reichert 1/3″ lens and a No. 4 ocular, with the exception of Fig. 2, *b* (1/6″ lens + 4 ocular), and Fig. 10 (1/6″ lens + 2 ocular, reduced to half-size). Figs. 6 and 10 have been reduced to one-half diameter, all others to two-thirds.

PLATE I.

FIGS. 1–4. 5-day plutei in $HgCl_2$, *n*/1,000,000.

FIG. 1. After 24 hours.

FIG. 1*a*. The most frequent type, with arms considerably resorbed, aboral end dilated. Anal view.

FIG. 1*b*. Similar, but arms more resorbed. The red pigment grains are here represented. Anal view.

FIG. 1*c*. Similar, but not quite so much arm-resorption; marked dilatation of the trunk-region. The epithelium of the trunk and aboral regions is exceptionally thin. Lateral view.

FIG. 1*d*. More complete dedifferentiation. The trunk and aboral regions are contracted instead of dilated; consequently the aboral ends of the spicules are not separated, and the trunk epithelium is thicker than normal. Anal view.

FIG. 1*e*. Similar, but with more pronounced arm-resorption. Anterior view.

FIG 2. After 72 hours.

FIG. 2*a*. Specimen with arms vestigial and dense, thin form, and clumping of mesenchyme. The stomach is normal, the intestine and œsophagus shrunken. Lateral view.

FIG. 2*b*. Aboral end of the same, under higher magnification. The light masses are composed of pale yellow cells with few granules. The dark bodies are red pigment. Quite aborally is a mass of slightly different clear cells.

FIG. 2*c*. The commonest type. The anal arms are totally absent, the oral arms and lobe almost entirely so. The skeleton of the oral arms is just protruding. The trunk and stomach are swollen, but the intestine contracted. Oblique anal view.

FIG. 2*d*. No trace of arms or of the form-differentiation of the oral end remains. On one side, the skeleton of the oral and anal arms has disappeared. The gut is shrunken, and its parts lie nearly in a straight line. Oblique anal view.

FIG. 2*e*. Complete dedifferentiation of the form of both oral and trunk regions. Body opaque and dense, filled with clumped mesenchyme. Skeleton reduced to two straight rods. Lateral view.

FIG. 3. After 96 hours.

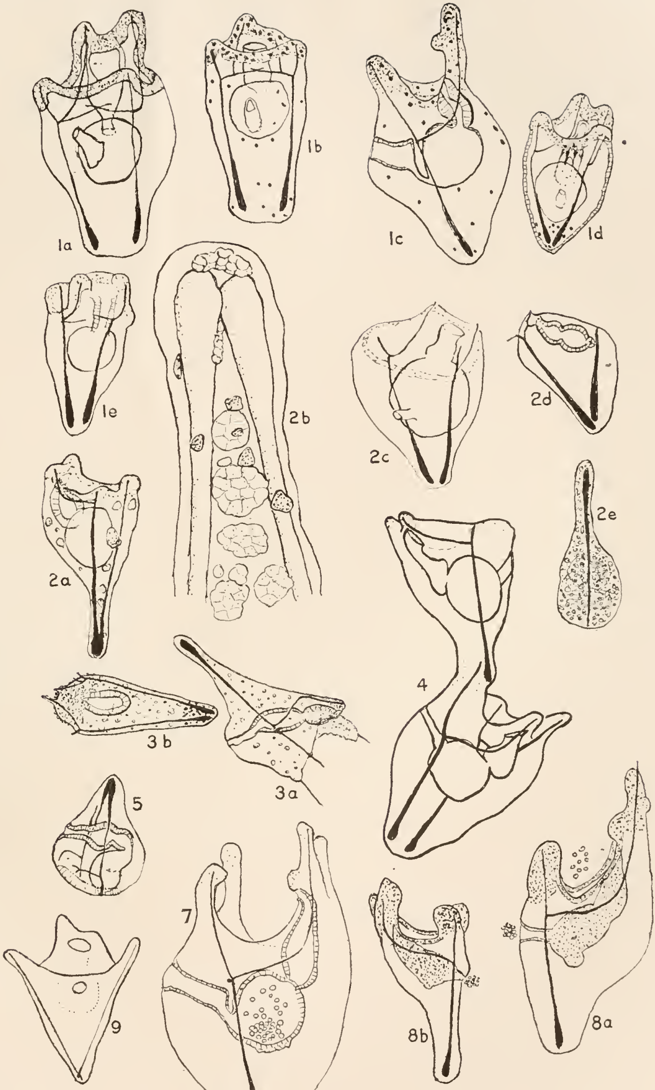
FIG. 3*a*. Protrusion of skeleton, disintegration of oral arms only. Trunk region still somewhat expanded. Lateral view.

FIG. 3*b*. Specimen dedifferentiated in all regions, with incipient death-changes. A mass of red pigment aborally. Disintegration of whole oral region beginning. Anterior view.

FIG. 4. A pair of plutei found grown together after 72 hours. Lateral view.

FIG. 5. A 4-day pluteus in $HgCl_2$ *n*/2,000,000, after 120 hours. Note the remains of the ciliated band, the reduced stomach but dilated œsophagus, and the abnormal skeleton. Lateral view.

PLATE I.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2e
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3a
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9
8b
8a

J. S. HUXLEY.

Figs. 6–8, and 14. 9-day plutei in $HgCl_2$ $n/1,000,000$.

Fig. 7. After 48 hours, in $n/1,000,000$ $HgCl_2$. The third pair of arms has disappeared, the other arms markedly reduced; note the clubbing of one of the anal arms. The stomach contatins numerous cells migrated out of its walls. Aboral and trunk regions are swollen. Lateral view.

Fig. 8. After 72 hours in $n/1,000,000$ $HgCl_2$.

Fig. 8*a*. The arms are markedly dense, and very small. The skeleton of the oral arms protrude slightly. The remains of the oral lobe are visible. Stomach and œsophagus are quite dense. Cells have been extruded from both anus and mouth. The trunk is slightly swollen. Lateral view.

Fig. 8*b*. More advanced stage. The oral lobe has disappeared, the arms are dense and shorter, the trunk and aboral regions are affected. Cells have been extruded at the anus. Lateral view.

Fig. 9. Wide-angled pluteus. 2 days from fertilization in KCN $n/100,000$. 2 days in sea-water. Only the anal skeleton is drawn. Anal view.

PLATE II.

FIG. 6. Outline of 9-day control pluteus when transferred to the solution $n/1,000,000$ $HgCl_2$. The skeleton is omitted. Anterior view.

FIG. 10. Differential disintegration of gastrula in $n/1,000,000$ $HgCl_2$. The nature of the spherical non-disintegrated mass was not ascertained.

FIGS. 11–15. Stages of progressive dedifferentiation of plutei after replacement in sea-water from toxic solutions.

FIG. 11. Form without trace of ciliated band. Gut epithelium cuboidal (occasionally contracting). Trunk ectoderm thin. 2 days in KCN $n/100,000$, 17 days in sea-water. m = mouth.

FIG. 12. Completely spheroidal forms.

FIG. 12*a*. With aboral ends of spicules broken. Gut straight, with cuboidal epithelium. No trace of ciliated band, oral lobe, or typical form. Some branched mesenchyme cells. m = mouth. 2 days in $HgCl_2$ $n/1,000,000$, 10 days in sea-water.

FIG. 12*b*. Another specimen from the same vessel, 3 days later. Faintly motile. Gut reduced to a closed vesicle with cells aggregated round it. A curious projection with sharp lobes in oral region.

FIG. 13. Extreme reduction.

FIG. 13*a*. Non-motile, with remains of aboral ends of spicules, a dense mass of cells surrounding a gut-vesicle, and very thin ectoderm. 1 day in KCN $n/25,000$, 13 days in sea-water.

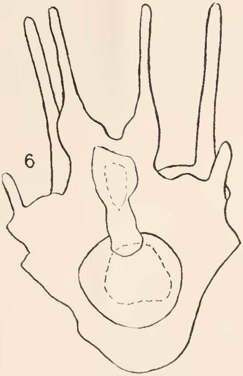
FIG. 13*b*. Still further reduction. Remains of only one spicule. Dense interior, with no visible trace of internal organs. 4 days in KCN $n/25,000$, 10 days in sea-water (with *Nitzchia*).

FIG. 13*c*. Faintly motile. No trace of spicules. Oral projection as in 12*b*. Several brown aggregations of cells, and a vesicle presumably derived from the gut. 2 days in $HgCl_2$ $n/1,000,000$, 30 days in sea-water (with *Nitzchia*).

FIG. 14. A larva not quite so much dedifferentiated as 12*a*.

FIG. 15. A much-reduced larva, showing protrusion of the left spicule at both ends. Where it protrudes aborally, a ring of thickened ectoderm surrounds it. The other spicule is absent; presumably it has simply fallen out. The œsophagus has partially disintegrated. 3 days in $HgCl_2$, $n/1,000,000$, 2 days in sea-water.

FIG. 16. A larva treated like that shown in Fig. 7.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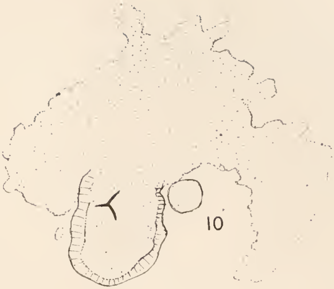
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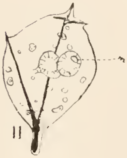

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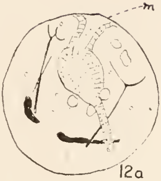

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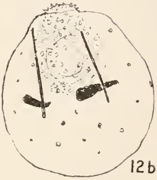
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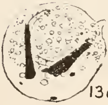
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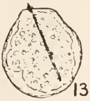
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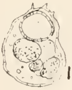
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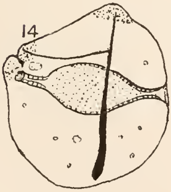
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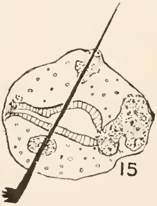
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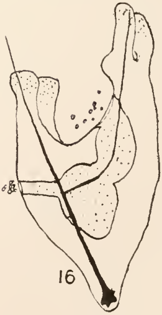
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J. S. HUXLEY.

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BIOLOGICAL BULLETIN

"REVERSAL OF INHIBITION" BY ATROPINE, IN CATERPILLARS.

W. J. CROZIER,

Zoölogical Laboratory, Rutgers College.

With a variety of invertebrate animals it has been found that strychnine exerts upon the neuromuscular structures an effect essentially similar to that which it displays in connection with the synapse-substance of vertebrate central nervous organs—namely, a decreased resistance to transmission, resulting in enhanced reactiveness; and a selective excitation of extensor elements, leading to opisthotonic curvature and to "reversal of inhibition" in responses involving reciprocal innervation. Representatives of the following groups are known to show behavior of this type under strychninization, and must therefore be accorded as a common property the possession of strychnine-receptive complexes suitably situated in their nervous organs: Platyhelminthia (*Planaria,*[1] *Bdelloura*[2]); Mollusca (*Chætopleura,*[3] *Chromodoris,*[4] *Limax,*[1] *Onchidium,*[5] *Loligo*[6]); Echinodermata (*Thyone,*[7] *Asterias*[8]); Annelida (earthworm,[9] leeches[1]).

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[1] Personal observation.

[2] Moore, A. R., *Jour. Gen. Physiol.*, I., p. 97, 1918.

[3] Crozier, W. J., *Jour. Gen. Physiol.*, II, p. 627, 1921.

[4] Crozier, W. J., and Arey, L. B., *Jour. Exp. Zoöl.*, Vol. XXIX., p. 261, 1919.

[5] Crozier, W. J., and Arey, L. B., *Jour. Exp. Zoöl.*, Vol. XXXII., p. 443, 1921.

[6] Moore, A. R., *Jour. Gen. Physiol.*, Vol. I., p. 505, 1919.

[7] Pearse, A. S., Biol. Bull., Vol. XVIII., p. 42.

[8] Moore, A. R., *Jour. Gen. Physiol.*, Vol. II., p. 201, 1920; *Jour. Pharm. Exp. Therap.*, Vol. IX., p. 167, 1916.

[9] Knowlton, F. P., and Moore, A. R., *Amer. Jour. Physiol.*, Vol. XLIV., p. 490.

Since strychnine has been relatively unique among neurophil substances in producing effects of the kind specified, it is desirable to call attention to the fact that among insects (caterpillars) phenomena identical in their nature with the reversal of inhibition typically induced by strychnine may be produced through the action of atropine; whereas, with these animals, strychnine is singularly ineffective and in fact fails to produce "reversal of inhibition." This point is important for the conception of progressive chemical differentiation of the nervous system in the animal series. Moreover, it raises certain difficulties in the way of employing strychninization as a test for the presence of synaptic structures or their homologues;[10] but further study is required to permit discussion of this aspect of the matter.

The experiments had in view the general impression that insects (and other arthropods) are peculiarly resistant to strychninization.[11] A common method of testing the action of a drug has been to submerge the organism in an appropriate solution, or to introduce the material into the alimentary canal. The cuticula of arthropods, however, may seriously interfere with such trials; its permeability is but little understood. Similarly, with *Ascaris* Schroeder[12] reported that immersion for three hours in 0.5 per cent. solution of strychnine was without effect; but some time ago I observed that characteristic squirming movements could be induced by injecting weak strychnine solutions into the interior of the body.

Resort was accordingly had to hypodermic injection. With small insects such procedure is impracticable; moreover, it was desirable to use forms in which the nervous system had not undergone great condensation. Suitable material was found in certain large caterpillars, of which five species were employed: *Protoparce celeus, Samia cynthia, S. promethia, Automeris io,* and *Ceratomia catalapæ.* The last named was not quite so well adapted to the injection as were the first four, upon which most of the observations were made. Half-cubic-centimeter volumes of solution

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[10] Parker, G. H., "The Elementary Nervous System," Philadelphia, 229 pp. 1920.

[11] Biberfield, J., *Ergebn. Physiol.,* Jahrg. 17, p. 175; and the fact is well known to economic entomologists.

[12] Schroeder, W., *Arch. Exp. Path. u. Pharm.,* Bd. XIX., 290, 1885.

could be injected without material loss of fluid through the puncture; sealing the puncture with celloidin, after withdrawing the injection needle, was found unnecessary, and indeed undesirable, as leading to local irritation. Strychnine injection was usually made laterally at about the level of the first prolegs; the needle, pointing anteriorly, was thrust through the cuticule of an intersegmental constriction. It may be stated at once that the level of the injection appeared not to influence the outcome.

Upon the injection of strychnine sulphate in concentrations less than 1 : 100 no efforts could be detected, beyond a momentary loss of the ability to creep; this was not specific and could be induced by injecting distilled water or Ringer solution. *Protoparce celeus* and other forms were observed to drink as much as 0.8 cubic centimeter or more of 1 per cent. strychnine sulphate.

In *Automeris io* injection of 0.5 c.c. of 1 per cent. strychnine sulphate led to twitching contractions of the abdominal musculature, sometimes symmetrical, sometimes better developed at one side; the legs and prolegs were quiescent, nonreactive to stimulation, and the prolegs particularly were pressed together. After 4 minutes a pinched proleg retracted its terminal comb, but no further response was observed; the body musculature was very "flabby." At about 10 minutes subsequent to injection there appeared a phenomenon, only partially reproduced with the other species, which is the most closely related among those observed to the "typical" strychnine effect. With the animal on its side, the body may be bent somewhat, either dorsally or ventrally. If the caterpillar be given a slight ventral flexure, rhythmic twitchings appear in the dorsal thoracic zone, which serve to make the body curvature distinctly concave dorsally; whereas, with a dorsal curvature initially impressed, the caterpillar remains in that position, although the dorsal twitchings persist. The twitchings of the dorsal muscle bands likewise continue when the larva is picked up by its posterior spine.

The stimulation of the contraction of dorsal muscle bands is similar to the stimulation of dorsal extensor muscles in other groups, but further evidence of characteristic strychnine action is lacking. It is probably significant that *Automeris io* is distinctly the most reactive, most "irritable," of the caterpillars studied.

With the other species employed in the experiments this effect was not seen. In each form used, however, strychnine in saturated solution (volume 0.5 c.c.) produced temporary but violent convulsive movements, incoördinated trembling movements of legs and prolegs, followed by quiescence, and often a rather strong *ventral* flexure and paralysis of the appendages. Twelve hours was sufficient to permit full recovery from the effects of even such doses of strychnine. Experiments with various aquatic larvæ (especially of *Psephenus*), and upon crayfish, further point to the very slight toxic effect of strychnine, and likewise show the detectable tendency to produce opisthotonic curvature. Crayfish, even when holes have been punctured in the cuticula, presumably facilitating absorption of the drug, were found to live for 9 days in a strychnine solution originally 1 : 1000 and slowly evaporated during the nine days that the test continued. The backward swimming of the crayfish is impeded by the strychnine, while there is maintained an incessant forward creeping.

In contrast with this behavior of the strychninized arthropod neuromuscular system, in annelids we observe typical "reversal of inhibition," although in the earthworm[9] it is perhaps more difficult to demonstrate than is true with leeches. Several species of leeches have been studied in dilute strychnine sulphate, and for present purposes the following findings may be cited: there is pronounced contraction of the dorsal longitudinal musculature, relaxation of the ventral, while in response to local irritation the customary behavior of longitudinal, transversal, and dorsoventral muscles is completely reversed by the drug.

Atropine.

Injection of 1 per cent. atropine sulphate, 0.5 c.c., produced in all forms studied an abrupt loss of creeping ability, within 1 minute of the injection; this was followed by a period of segmental tremblings, involving some ventralward contraction (the animals lying on the side), and within 5 minutes after injection there appeared a very striking reversal of the behavior of the prolegs. Normally, and especially, in their natural use, when the creature creeps on the edge of a leaf, slight tactile stimulation of the ventrum between the legs suffices to induce the extension of the terminal combs of the prolegs, simultaneous extension of the proleg pair, and their apposition—the whole movement giving an effective embrace of a leaf margin. The muscles governing the extension of the terminal combs, those having to do with the movements of the prolegs themselves, and the muscles of the body wall,[13] all are involved in this coördinated movement of response to stimulation of the ventral body wall; and under atropine the movement is entirely reversed — combs are retracted, prolegs greatly retracted, body wall itself retracted rather than, as normally, protruded in the region between the proleg. This "reversal" was found only in connection with the prolegs, never with the thoracic legs.

Normally, lightly touching a proleg comb, or the skin between the prolegs, leads to extension of both members of the pair; then a subsequent light touch leads to the usual clasping motion; under atropine, touching comb or skin between prolegs results in the spreading apart and deep retraction of the prolegs. The reversal of the proleg response persisted for 20 hours or more.

Atropine is very effective in producing heightened irritability; and usually there was evidence, also, of a tendency to dorsal contracture—slight suggestion of an opisthotonic state (perhaps related to the lifting of the head when the normal animal is disturbed on a leaf).

In one or two cases transitory evidence of proleg reversal was obtained during observation of larvæ injected with nicotine solutions, and particularly with pilocarpine; it could not be produced at will with these substances, however, nor was trace of the phenomenon detected under treatment with a variety of other neurophil drugs. It is a characteristic and pronounced effect peculiar, in my experience, to atropine.

With strychnine, and less clearly with atropine, suggestion was had of the special stimulation of the dorsal muscles ("opisthotonus"). This effect is not specific, or at least is not so specific as the proleg reversal. Tetraethylammonium chloride (*m*/64) invariably led to striking opisthotonic spasms, due chiefly to contraction of dorsal muscles at the thoracic level. Chloroform (one third saturated) behaved somewhat in the same way, but more vaguely. Ventralward contraction, especially at the anterior end, was always produced by nicotine, camphor, phenol, resorcin, and caffeine.

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[13] Cf. Peterson, A., *Ann. Ent. Soc. Amer.* Vol. V., p. 246, 1912.

Chemical Differentiation.

It is of interest to make note of certain facts bearing upon the question of phylogenetic neuromuscular differentiation, from the standpoint of reactivity to drugs.

The larvæ used agree in showing that the following instances exert a very definite neuromuscular excitation—evidenced by spontaneous writhings, often of definite pattern, or by temporarily heightened responsiveness, or both:

* nicotine	(1:500)
* picrotoxin	(1:240)
* camphor	(saturated)
* pilocarpine	(1:150)
* adrenaline	(1:1000)
phenol	(1:2000)
* resorcin	(1:1000)
caffeine	(saturated solution; 1:200, without effect)
* tetraethylammonium hydroxide	($m/64$)
chloroform	(1/3 saturated)
* atropine	(1:500)
strychnine	(1:100)

* (Especially exciting.)

Creatine, $m/8$, gave only slight temporary paralysis, probably not specific.

These facts suggest obvious differences when the behavior of the caterpillars is compared with that of other invertebrates. The action of atropine, and the relative lack of effectiveness of strychnine, point to parallelisms with conditions in cœlenterates,[14] rather than with forms closer akin. Crayfish injected with strychnine fail to yield evidence of "reversal" in the use of muscle groups, and atropine has a marked effect in stimulating the thoracic ganglia. But in how far the peculiarities here revealed are arthropodan characteristics cannot as yet be said.

In comparing a series of larvæ, such as the *Samias* and *Io,* one is struck by the fact that the *Io* caterpillar, provided with urticant

[14] Moore, A. R., *Proc. Nat. Acad. Sci.,* Vol. III., p. 598, 1917.

spines, shows normally a rather violent, jerky response to even mild stimulation. This is obviously a point of some significance, ethologically, since it increases the effectiveness of the "hairs" in penetrating and stinging. But under appropriate drug injection the quick, "snappy" type of behavior rather peculiar to *Io* can be easily brought about, for example, in a *Samia* caterpillar. Pilocarpine gave especially instructive comparisons of this sort. The sensory thresholds are so lowered by this substance that a *Protoparce* or *Samia* twists sharply in response to a single light touch, contracting at the place of stimulation, just as *Io* normally does. Biting reactions are likewise enhanced, so that *Samia* snaps savagely at its own spines encountered in writhing, or at forceps touching the head, much as *Io* normally does. The production of behavior normal to one species in another form characteristically more sluggish shows that the drug produces no *new* forms of response, but merely accentuates types of reaction for which the structural pathways already exist. Some light is thus given on structural basis of behavior differences in related forms.

The action of atropine shows clearly that reciprocal innervation exists in these insects, at least so far as concerns the action of the antagonistic muscle groups of the prolegs and walls of the segments. That strychnine, even in high concentration, fails to react in its characteristic manner with the synapses necessarily involved in these reciprocally acting nervous elements, shows that strychnine may fail to reveal the presence of synapses; and also that synapses even in types so closely related as annelids and arthropods may differ from one another;[15] or else that the "reversal" phenomena induced by atropine are brought about through action of the latter upon a locus distinct from that usually reacting with strychnine.

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[15] Cf. Cushney, A. R., *Science*, N.S., Vol. XLIV., p. 482, 1916. It may be suggested that the slight acidity of caterpillar hæmolymph (p_H 6.8–6.6; Jameson and Atkins, *Biochem. Jour.*, Vol. XV., p. 209, 1921) might influence the action of the drugs; but I doubt that the low toxicity, at least, of strychnine can be explained in this way.

NOTES ON SOME PROBLEMS OF ADAPTATION: 9. CTENIDIAL VARIATION IN CHITON.[1]

L. H. SNYDER AND W. J. CROZIER,
(1901–) (1892–)

ZOÖLOGICAL LABORATORY, RUTGERS COLLEGE.

1. The respiratory organs of the Amphineura comprise a more or less extensive row of branchial filaments on either side of the body, in the groove between girdle and foot. Within the species it is known that the number of these ctenidia is variable. It is likewise known that the right and left ctenidial sets of an individual may differ in number of included gills. Published observations on these points have been summarized by Pelseneer (1920, p. 201). It appears that in some species the number of ctenidia may increase with the size of the animal; in others it seems to decrease; while in still others no correlation is very evident between size of animal and number of gills. We have sought to determine the nature and extent of ctenidial variation in *Chiton tuberculatus* of the Bermudas, having in mind the possibility of evidence upon the following points:

(1) Ctenidial variation as correlated with size (age),
(2) with sex, and
(3) with habitat; and
(4) indications of asymmetry.

The gill plumes were counted in 100 individuals, of a range of sizes. The length of the fourth shell-valve, along its mid-line, was measured as an index of size. By means of graphs such as that given by Arey and Crozier (1919, p. 164) the length of the fourth valve may be translated into terms of the length of the individual; the curled condition of many of the specimens, as preserved, precluded direct determination of their lengths. It is sufficient to note that the length of the shell-valve varies directly with the total size of the animal, though the relation is not exactly a linear one, owing to the effects of erosion and its consequences (Arey and Crozier, 1919).

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[1] Contributions from the Bermuda Biological Station for Research. No. 141.

The total number of ctenidia is at first 64–66, and increases in a fairly regular manner as growth proceeds. The average maximum number is about 98. The data are given in Table I.

TABLE I.

Total number of branchii	Length of valve 4 in mm. 0	1	2	3	4	5	6	7	8	9	10	11	12	13	
64-66	2														2
67-69															
70-72															
73-75	1	1													2
76-78		1	1												2
79-81		1	1	1											2
82-84			2	1	2	1									6
85-87		2				1						1			4
88-90				2	4		1	1	1						9
91-93			1	2	4	6	2	1			1		1	1	19
94-96			3		1	4	1	1	2	3		1			16
97-99						1	2	10	2		1		1		17
100-102						2	1	4	4			1	1		13
103-105								1	3						4
106-108							1	1				1			3
	3	5	8	6	11	15	8	19	12	3	2	4	3	1	100

DISTRIBUTION OF TOTAL NUMBERS OF CTENIDIA IN RELATION TO LENGTH OF FOURTH SHELL-VALVE; ALL INDIVIDUALS.

2. Since the number of gills is determined in part through growth, it is of interest to learn if the extent of the gill series is influenced by sex. Fig. 1 makes it plain that such influence, if real, must be slight. It is possible that there is on the average a slightly higher number of ctenidia in the males, when the comparison is made of males and females equal in size; on the basis of *age* this slight difference is increased, for the females are the larger, at the same age (unpublished data). It is doubtful if this

difference can be correlated with relative activity. New gills are in all probability added at the posterior end of the ctenidial series. Occasionally gill plumes are observed with divided tips (such as have been figured by Pelseneer, 1920, p. 204).

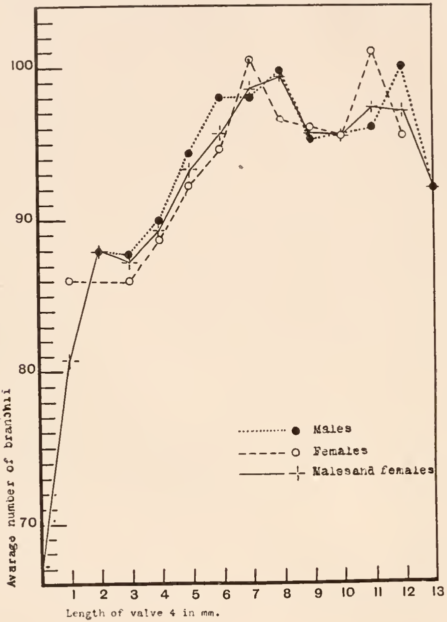

FIG. 1.

It appears from Fig. 1 that the full number of ctenidia is established before maximum growth is attained. It is unlikely that there is a real *decrease* in the number of ctenidia with extreme age (cf. Table I.). The number of gills is frequently mentioned as a subsidiary character in descriptions of species, hence recognition of the variation we record may be of taxonomic importance.

3. This inquiry began with the thought that the total number of gills might be influenced by habitat. The number of individuals at our disposal is small, but sufficient for preliminary study of the point.

Our *Chiton tuberculatus* were secured from two well-contrasted situations (cf. Crozier, 1918): an exposed ocean beach on the south shore of Bermuda (here referred to as "South Shore"), and the shores of an enclosed sound, Great Sound. In Fig. 2 the mean ctenidial frequency for each size class is contrasted for these two locations. Clearly there is no great difference between the two sets of observations, though the lower number of ctenidia in the younger animals from the South Shore may perhaps prove significant.

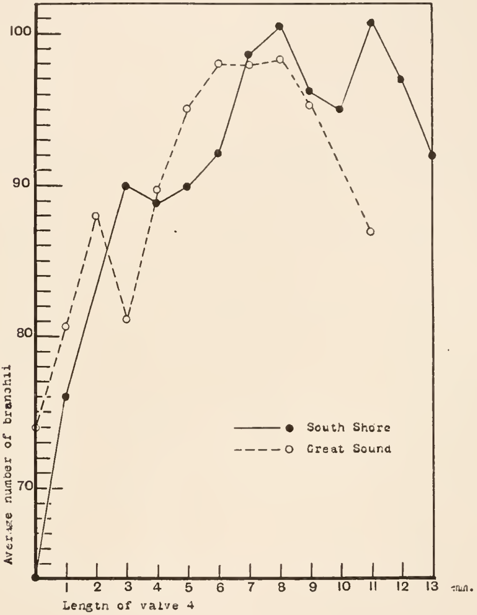

FIG. 2.

4. Is it possible that the asymmetry of gasteropods is in some manner foreshadowed among the chitons? The two liver-lobes of young chitons are at first symmetrical, but later the right lobe becomes the smaller and is pushed anteriorly (Pelseneer, 1906,

p. 44); it has been noted that the number of the auriculo-ventricular connections of the heart may be dissimilar on the two sides (Pelseneer, 1897, p. 23); several instances of valve-fusion seemed asymmetrical (Crozier, 1919); and the existence of minor asymmetries in the two gill series has been recorded several times (Pelseneer, 1897; 1920, p. 201). But the published data fail to cover the possibility of definite correlation with superior growth on the right or on the left side.

Our counts show that the distribution of asymmetries of the ctenidia follows a purely random course; the absolute amount of asymmetry, moreover, is small. When excess of gill-plumes on the right side as compared with the left is reckoned as *plus*, on the left side as compared with the right as *minus*, a frequency curve is obtained (Fig. 3) indicating an essentially chance distribution. It follows that the asymmetry is merely an accident of growth, giving a measure of the independence of the two sides in forming new gills. On this view, the extent of ctenidial asymmetry should increase, as a rule, with size. Table II. shows that this is the case.

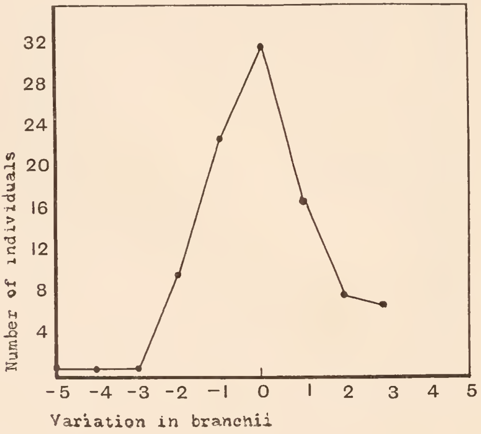

FIG. 3.

TABLE II.

RELATION OF ASYMMETERY (EXCESS NUMBER OF GILLS ON ONE SIDE) TO SIZE (CLASSES ACCORDING TO LENGTH OF VALVE 4.)

	Class Centers.			
	0.2 mm.	0.5 mm.	0.8 mm.	0.11 mm.
0.........	12	5	7	5
1.........	4	19	12	4
2.........	2	8	7	1
3.........	1		5	1
4.........			1	
5.........		1		

There is no apparent correlation of asymmetry with sex or with habitat. In the majority of cases the number of gill-plumes differs on the two sides, but the difference is usually not more than one. The asymmetry can therefore hardly be connected with the torsion of the body in gasteropods.[2]

Summary.—In *Chiton tuberculatus* at Bermuda the number of ctenidia increases from 32 on either side, in individuals about 1.0 cm. long, to an average of 49 on a side in individuals of the largest size (9–10 cm. long). The increase in the number of gills is at first quite rapid, so that the maximal number is achieved before the animal is 6.0 cm. long. The mean number of ctenidia is a little less in females than in males of the same size or age.

On an exposed ocean beach these chitons have the same number of gills as found in the population of an inclosed sound.

Minor asymmetries in number (1 to 5) are the rule when the two gill series of an individual are compared. Asymmetry follows a random distribution; it is an accident of growth and has no morphological significance.

CITATIONS.

Arey, L. B., and Crozier, W. J.

'19 The Sensory Responses of *Chiton.* Jour. Exp. Zoöl., Vol. 29, pp. 157–260.

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[2] Two instances were found of a curious malformation, which may be noted here. At the level of the fourth valve a tough "bridge" of tissue had grown across from the lateral wall of the foot to the girdle, forming an arch over the ctenidial channel. These "bridges" were about 3 mm. wide.

Crozier, W. J.

'18 Growth of *Chiton tuberculatus* in Different Environments. Proc. Nat. Acad. Sci., Vol. 4, pp. 325–328.

'19 Coalescence of the Shell Plates in *Chiton*. Amer. Nat., Vol. 53, pp. 278–279.

Pelseneer, P.

'97 Sur la morphologie des branchies et des orifices rénaux et génitaux des chitons. Bull. Sci. Fr. et Belg., tom. 31, p. 23–30.

'06 Mollusca; in: Lankester's Treatise on Zoölogy, Pt. 5, 355 pp.

'20 Les variations et leur héréditié chez les Mollusques. Mém. Acad. Belg., Cl. Sci., sér. 2, tom. 5, 826 pp.

CHROMOSOME STUDIES ON THE DIPTERA. IV. INCOMPLETE SYNAPSIS OF CHROMOSOMES IN *DASYLLIS GROSSA* FABR.

C. W. METZ,

Carnegie Institution of Washington, Department of Genetics.

Introduction.

In the robber fly, *Dasyllis grossa,* synapsis of chromosomes in spermatogenesis (and possibly in oögenesis) appears to be partially inhibited, in such a manner that three of the pairs of chromosomes undergo synaptic association for only a portion of their length. The behavior is relatively uniform and constant in these particular chromosomes and indicates that different regions of a chromosome behave differently as regards synapsis. The present account deals primarily with this feature of chromosome behavior and with its possible bearing on cases of abnormal cross-over values in genetical experiments with *Drosophila.*

The description of spermatogenesis is taken largely from one specimen of *D. grossa* Fabr., kindly identified by Mr. C. W. Johnson; but it applies in a general way to all of the *Dasyllis* material I have studied (a dozen or more specimens, representing *D. grossa* Fabr., *D. thoracica* Fabr., and probably two or three more species). I am not certain that all of these agree in detail, but each one shows indications of incomplete synapsis in one or more pairs of chromosomes.

Dasyllis provides particularly favorable material for a study of chromosome behavior during spermatogenesis, because of the small number and relatively large size of the chromosomes; the large size of the nuclei; the relatively enormous number of cells present, representing all stages from early spermatogonia to spermatozoa; and particularly the serial orientation of successive stages throughout the tubular testes, making it easy to trace the chromosome behavior step by step through the growth period. In addition, it should be mentioned that there is no contraction or synizesis stage, and no true "diffuse" stage when the chromatin can not be seen.

In its general features spermatogenesis in *Dasyllis* resembles that in *Asilus sericeus,* as described by Metz and Nonidez ('21), and no attempt will be made to give a complete account here. The technique employed in the present work was the same as that described in the preceding paper. The material was fixed in strong Flemming and stained in Heidenhain's iron hæmatoxylin.

Dasyllis is unique among the Diptera thus far studied in possessing an unpaired X-chromosome in the male. This is true of all the material I have studied in the genus. The other Diptera have all been of the X-Y type. In an earlier paper (Metz, '16, p. 243) *Dasyllis* was tentatively considered to possess a Y-chromosome, but additional material has shown clearly that no Y is present unless it is so minute as to be practically invisible.

I am indebted to Miss M. S. Moses for assistance in the laborious work of examining the ovaries in connection with the observations on oögenesis, and to Miss Ruth Lincks for making the drawings for the accompanying figures.

Spermatogonia.

The spermatogonial chromosome group of *D. grossa* consists of three pairs of V-shaped and one pair of J-shaped (atelomitic) autosomes, and the unpaired X, as shown in Fig. 1. The J-shaped pair appears straight in this figure, but in later metaphases its subterminal spindle fiber attachment is evident. The V-shaped pairs are of different sizes, the smallest being readily distinguishable from the other two. Thus the X and two of the autosome pairs may be identified individually.

As in all of the other Diptera that I have studied, the paired association of chromosomes is persistent through successive spermatogonial generations, and into the final spermatogonial anaphase. In spermatogonial prophases (Figs. 2 and 3) the pairing of the large chromosomes does not seem to be as intimate medially as in most other flies—a fact which suggests that the peculiar repulsion or lack of attraction exhibited in the growth period is existent here also.

The Spermatocyte Growth Period.

In *Dasyllis* the spermatocyte nucleus is relatively large at the beginning of the growth period; and partly on this account, perhaps, the growth period does not involve an increase of more than about 30–50 per cent. in nuclear diameter.

The description of the growth period may best be taken up at a point shortly after growth has begun (returning later to a consideration of the earliest stages). At this time the chromosomes, with the exception of the condensed X-chromosome, are in the form of long, deeply staining threads, closely applied to the nuclear membrane. There are four pairs of threads, in one of which (apparently the smallest) the two members are usually closely associated throughout their length—*i.e.,* the synaptic association is complete. In each of the other three the association is evident at each end but toward the middle the two components diverge to form large loops. Each pair of threads is very long, frequently extending more than half way around the circumference of the nucleus, hence it is practically impossible to represent them all in one figure. Individual pairs, however, are shown in Figs. 7 to 10. Most of these are complete, but in some (*e.g.,* Fig. 9) one or both ends of a pair may be cut off. To one of the three looped pairs (apparently the largest) is attached a large dense body (Fig. 7), which serves to identify this pair throughout the entire growth period. This body (see page 258 for description) is attached to both members of the pair in the looped region and normally lies at a point near one end of the loop—a position which it occupies with surprising regularity.

The three large pairs of chromosomes at this stage give the appearance of having undergone synapsis only near their ends—the threads having remained well apart medially for about one third to one half their length. In the smallest pair, as noted above, synapsis is usually complete, but in a few cases a small loop is present. The chromosome on the left in Fig. 9 appears to be such a case. Throughout the remainder (80 per cent. or more) of the growth period the condition of the chromosomes is maintained with relatively little change. The cells and nuclei grow somewhat and the chromosomes become more condensed and hence more easily examined. The X-chromosome remains condensed throughout. Successive stages are represented by thousands of cells and the transformation during growth is so gradual that scarcely any change is observable from one cyst to another down the testis. It seems certain, therefore, that no important stage has been overlooked in this region.

Figs. 11 and 12 represent entire, or almost entire, nuclei showing the condition maintained by the chromosomes up to the end of the growth period. In each of the three large pairs of chromosomes the two components diverge medially in the form of a loop; while in the shorter pair they are usually closely applied throughout their length, with only occasionally a small loop visible. In Fig. 11 the entire contents of the nucleus are drawn in position; in Fig. 12 the pairs are transposed to facilitate examination, but are all taken from the same nucleus.

During the late prophase, as the chromosomes are about to go on the spindle, they become so condensed and shortened that in many cases the loops become closed (Fig. 13). The line of separation between the two chromosomes, however, is still evident, and it is practically certain that no intimate association occurs here.

It appears, then, that in the case of the three large pairs of chromosomes synapsis has been incomplete, unless it occurred at a very early stage in the growth period and was followed by a secondary opening out to form the loops. The evidence from the early stages may now be examined from this point of view.

The Early Growth Period.

The early growth stages resemble those of *Asilus sericeus* (Metz and Nonidez, '21) in a general way, but the details differ materially. In *Asilus* homologous chromosomes become closely associated in the final spermatogonial telophase and remain thus as they draw out into threads. Just before they draw out (stage *b*) the pairs look like single chromosomes relatively clear cut in outline. In *Dasyllis* the chromosomes are likewise paired; but the pairs form loose, irregularly granulated aggregates, giving little or no indication of the intimate association seen in *Asilus*.

The aggregates become more loose and irregular in structure as growth proceeds, and then draw out into irregular, granulated threads. Only two bodies remain condensed: one the X-chromosome and the other the "dense body" attached to the large pair of chromosomes. As the aggregates spin out into threads a more intimate association becomes possible. Unfortunately as the spinning out proceeds the threads become entangled and lose much of their staining capacity, making this the most difficult stage to analyze. In fact, it is impossible to trace all of the chromosomes in any one nucleus. It is significant, however, that as soon as the aggregates have elongated two kinds of threads may be observed: single and double; and occasionally in favorable nuclei the two members of a double one may be seen to diverge in the form of a Y, or may even form a loop essentially like those of later stages. The most convincing evidence as to the nature of events at this time is obtained from an examination of the threads attached to the "dense body." As has been noted above, this body in later stages is attached to the two single threads making the loop in one of the large chromosome pairs. By following this structure through the early stages, then, it should be possible to determine whether or not the loop is present here also. A careful study of this feature has convinced me that the loop is normally present throughout the early stages—from the time the chromosomes first elongate. In some cases the entire structure may be seen, as shown in Figs. 4 and 6, and in others it is evident that the threads running out from the dense body are single, not double. The fact that single threads and occasionally loops (Fig. 5) may also be seen in other parts of the nucleus makes it seem almost certain that the same conclusion applies to the other large chromosomes. It is practically impossible to differentiate the smallest chromosome pair from the others in the early stages, hence I have been unable to determine whether or not it possesses a loop at this time. A little later the chromosome pairs move to the periphery of the nucleus and become separated sufficiently to permit of individual analysis—which brings them into the stage with which our description began (Figs. 7 to 9).

It is possible, of course, to assume that the chromosome pairs do not behave synchronously in the very early stages, and to imagine them undergoing, one at a time, a complete synapsis followed immediately by a partial opening out into loops. This would account for the constant presence of both single and double threads in the nucleus. Such an explanation seems improbable, however, from analogy with other forms, and especially in view of the evidence furnished by the chromosome pair attached to the dense body, which may be traced through all the stages. The conclusion seems justified, therefore, that synapsis does not occur in the looped regions of the three large chromosome pairs.

THE "DENSE BODY."[1]

This body, which appears to take the place of the nucleolus in other flies, has such a consistent connection with one of the large pairs of chromosomes throughout the growth period that its structure and history may be considered in some detail. It is usually ovoid or spherical, deeply staining in iron hæmatoxylin, and clear cut in outline. In some cases it is clearly bipartite in structure, and rarely it is divided into entirely separate parts. As already noted, it is attached to both members of one of the large chromosome pairs, probably the J-shaped pair. When it is divided into two parts, as in Figs. 17 to 19, this independent attachment is shown clearly. Associated with the dense body, on the side opposite the attachment to the chromosomes, is an achromatic structure (presumably a plasmosome), of variable size and irregular outline, as shown in Figs. 11, 12, and 18.

The origin of the dense body can not be traced accurately enough to demonstrate that it arises directly and equally from the two members of the one pair of chromosomes, but its subsequent history (attachment, bipartite structure, behavior in late prophase) suggests such an origin.

During the prophase of the first spermatocyte division, as the chromosomes go on the spindle, the dense body diminishes in size. It gives the appearance of being taken up, in part at least, by the attached chromosomes, for there is little indication of any of it diffusing or breaking off. It separates into two components at this time, coincident with the separation of the two chromosomes (Figs. 20, 21) preparatory to the reduction division. They persist in this condition up to, and perhaps through, the metaphase, as do the somewhat similar "chromosome vesicles" observed by Carothers ('13) in the Orthoptera *Brachystola* and *Arphia*. I have not attempted to follow their history beyond this point.

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[1] Since the chemical nature of this body is not known it has been thought better to apply a descriptive term to it, rather than to call it a nucleolus, karyosome, or chromosome vesicle, each of which it resembles in certain respects.

The Maturation Divisions.

No special interest attaches to these and they will be passed over rapidly. The first division is reductional for autosomes and X-chromosome. The former appear as dyads with little evidence of a tetrad structure. Such a structure probably exists, however, and further extraction would perhaps bring it out, for in early anaphase each component of the dyad is itself clearly double. The X-chromosome goes to one pole in the first division and divides in the second. Metaphases of the first division and of the two types of second division are shown in Figs. 14 to 16.

Oögenesis.

Knowing the unusual behavior of the chromosomes during spermatogenesis, it would be of especial interest to determine whether or not similar phenomena occurred during oögenesis, but this has proved to be a very difficult task. Owing to the nature of oögenesis only a few, rather widely separated stages can be found in any one ovary, and the story has to be pieced together from an examination of many specimens. The orientation of stages, especially the early ones, is very difficult. Consequently I am only able to record a few observations at this time.

The chromosome group of the female, as expected, consists of five pairs—differing from that of the male only in the presence of two X-chromosomes instead of one (Fig. 22).

Near the apex of the ovarian tubules, in the region of transition from oögonia to oöcytes and nurse cells, are found nuclei with chromosomes such as those represented in Fig. 23 (probably oögonial) and in Figs. 24 to 28. In the latter very obvious loops are present, bearing a strong resemblance to those in the spermatocytes. These chromosomes are not pulling apart on the spindle, as their outlines might suggest, but are in resting nuclei, or at least nuclei that are not actively preparing to divide. Whether they represent the final generation of undifferentiated ovarian cells or represent an early growth stage of oöcytes and nurse cells, I am unable to determine. If the latter, then they may correspond to spermatocytes in the aggregated stage *b*. In any case, the condition seems to parallel that found in the male, for I have not observed such loop structures in any other Diptera. This gives some ground for expecting similar synaptic phenomena in the two sexes in regard to the feature with which we are concerned here.

After the growth period is well under way and the oöcytes are clearly differentiated from the other cells, the chromatin appears in the form of large, irregular, heavily granulated aggregates whose structure I am unable to analyze. Before this, however, there appears to be a diffuse stage in which scarcely any chromatin is visible in the nucleus. My present material is not adequate for a detailed study of these stages and their treatment must be deferred.

Discussion.

The above facts, it is believed, provide strong support for the conclusion that, in the male *Dasyllis* at least, synapsis is not uniform throughout the length of the chromosomes, but may, in the case of certain chromosomes, occur only in the terminal regions, leaving the homologous members separated near the middle.

So far as I know, no equally clear cases of this sort have been described before, although figures that suggest a similar condition are given by Mohr ('16, Figs. 85–90) for *Locusta viridissima,* by Robertson ('16, Fig. 163) for *Chortippus curtipennis,*[2] and by Wenrich ('16, Figs. 73–78) for *Phrynotettix magnus.* These are interpreted by the authors as cases of delayed synapsis (*Locusta* and *Phrynotettix*), or early separation of threads after synapsis (*Chortippus*); but it is possible that with further study some of them may prove to be cases of incomplete synapsis. Professor McClung informs me that similar conditions may exist in other Orthoptera on which he has made preliminary observations.

It has also been suggested to me by McClung that the absence of synapsis in the median part of the long J- and V-shaped chromosomes in *Dasyllis* may be due to these chromosomes each being compounded of two rod-like chromosomes united end to end, as he has found chromosomes to be united in *Hesperotettix* (McClung, '17). This offers an attractive lead toward an explanation, but it is necessary to assume some other influence as well, else all compound chromosomes should show this behavior. The latter influence seems to lie deeper in the chromosome organization. It is not revealed, however, by any morphological feature such as a difference in length or structure of the two components of a loop. Genetically one might assume the presence of balanced lethals to account for the results; but there is no evidence of their being present, and no chance of securing such evidence, because the flies are unsuitable for genetical study.

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[2] I am indebted to Prof. E. B. Wilson for calling my attention to the former, and to Prof. C. E. McClung for calling my attention to the latter case.

It is to be noted that the looped region of the chromosomes in *Dasyllis* is usually median. In many organisms the middle of the chromosome is apparently the last part to undergo synapsis, which suggests that the process in *Dasyllis* is actually an incomplete but otherwise normal synapsis. On the other hand, in the Diptera thus far studied, the synaptic process appears to be very different from that in other organisms, and there is no indication of synapsis beginning at the ends of the chromosomes and progressing toward the middle. Nevertheless, the fact that three pairs of chromosomes are affected similarly suggests an influence on the general synaptic process in these organisms, rather than the independent action of agents located in the respective pairs.

The chromosome behavior in *Dasyllis* recalls the genetical behavior of certain strains of *Drosophila melanogaster* in which crossing-over is greatly diminished in certain parts of linkage groups (Muller, '16; Sturtevant, '19; Detlefsen, '20). This decrease or elimination of crossing-over in part of a chromosome is just what one would expect from an incomplete synapsis such as described above.

However, it will be necessary to get both the genetical and cytological data from one organism before the evidence becomes satisfactory, and I do not wish to push the present analogy. In *Drosophila* crossing-over occurs only in the female. If this is true in *Dasyllis,* then oögenesis rather than spermatogenesis must be used for comparison. It also appears from the *Drosophila* data that many, if not most, of the cross-over modifications manifest themselves only in flies heterozygous for the cross-over "genes." In other words, as Sturtevant has suggested (p. 329), they appear to be due to an unlikeness in homologous chromosomes. In *Dasyllis* there is at present no evidence that the homologous chromosomes differ in the regions where synapsis fails to occur.

The cases involving different cross-over values in homozygous strains of *Drosophila* (C^{II} of Sturtevant, and unpublished data kindly furnished by Dr. Bridges) provide a closer analogy. At present it can only be said that the chromosome behavior in *Dasyllis* illustrates a type of mechanism which should give results similar to those observed in *Drosophila*.

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EXPLANATION OF PLATES.

All figures were drawn from sections 5*u* in thickness, with the aid of a camera lucida. Magnification approximately 3,000 diameters.

Plate I.

All figures except numbers 1 and 2 are from *Dasyllis grossa;* numbers 1 and 2 are from specimens not yet identified as to species.

Fig. 1. Spermatogonial metaphase.

Figs. 2 and 3. Spermatogonial prophases.

Fig. 4. Very early growth stage, only one aggregate or chromosome pair represented.

Fig. 5. Same stage, representing portion of another aggregate.

Fig. 6. About the same, or slightly later stage, showing two aggregates.

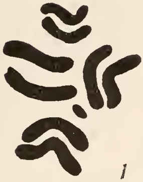
Fig. 7. Slightly later stage after the chromosomes have moved to periphery of nucleus. One chromosome pair and attached dense body represented.

Figs. 8 to 10. Similar stages showing other chromosomes. The chromosome pair on the left in Fig. 9 is presumably the small pair which usually has no loop or a very small one.

Fig. 11. Later stage, about middle of growth period. Entire nucleus represented, including the four pairs of autosomes and the small, condensed X.

Fig. 12. Later stage, entire nucleus; chromosomes transposed to facilitate examination.

PLATE I.



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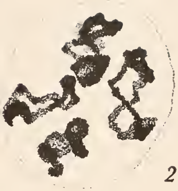

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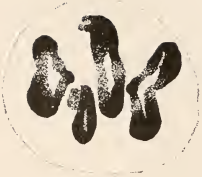

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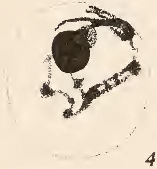

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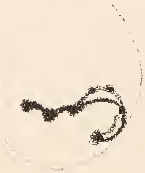

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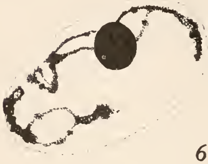

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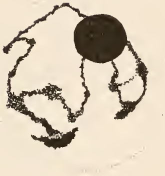

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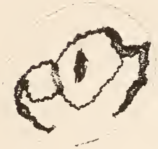

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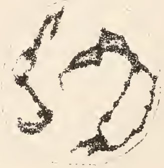

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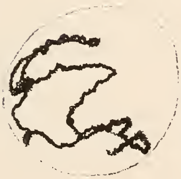

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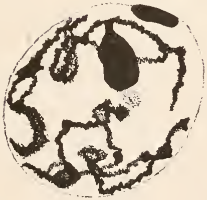

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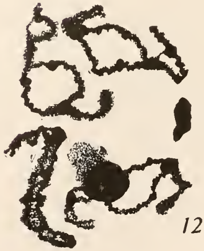

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C. W. METZ.

PLATE 2.

FIGS. 13 to 21. ♂ *Dasyllis grossa;* Figs. 22 to 28 ☿ *Dasyllis,* species not yet determined.

FIG. 13. Late prophase, first spermatocyte.

FIG. 14. Metaphase, first spermatocyte.

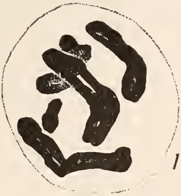
FIGS. 15 AND 16. Metaphases, two types of second spermatocytes, the former with X., the latter without it.

FIGS. 17 TO 19. Examples of the rare cases in which the dense body is represented by two separate smaller bodies.

FIGS. 20 AND 21. Examples of the chromosome pair with dense body attached, in late prophase.

FIG. 22. Ovarian cell (oögonium?), prophase showing loose association of middle region of large chromosomes.

FIGS. 24 TO 28. Chromosomes from oögonia, or early growth stage of oöcytes or nurse cells, showing median loops resembling those in the male.



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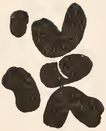

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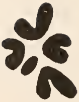

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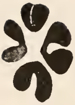

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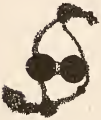

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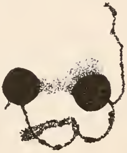

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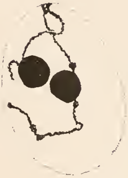

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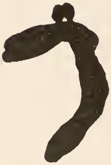

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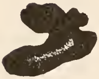

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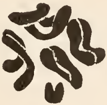

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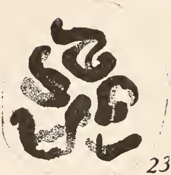

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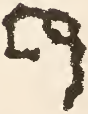

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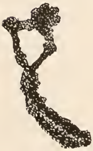

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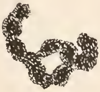

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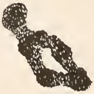

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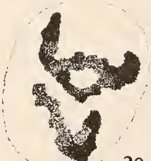

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C. W. METZ.

ISO-AGGLUTINATION AND HETERO-AGGLUTINATION OF SPERMATOZOA.

MYRA M. SAMPSON,

Department of Zoölogy, Smith College, Northampton, Mass.

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I. INTRODUCTION.

The agglutination of spermatozoa reported by Buller (1900) was first adequately described by Lillie (1913). The latter distinguished two types of agglutination differing from each other in cause and in characteristics: *iso-agglutination* produced by a substance secreted by ripe ova of the same species, and *hetero-agglutination* by substances present in egg secretions and body fluids of foreign species, Lillie (1914). The characteristics of these types will be considered in the following pages. In this report evidence is given for the first time of the occurrence of iso-agglutination in the black chiton, *Katharina tunicata,* and of hetero-agglutination between *K. tunicata* and *S. purpuratus,* and between *K. tunicata* and *S. franciscanus,* and the reciprocal hetero-agglutination between either *S. purpuratus* or *S. franciscanus* and *K. tunicata.* Similar reciprocal hetero-agglutination occurs between either *S. purpuratus* or *S. franciscanus* and *Ishnochiton magdalenensis.*

The results embodied in this report were obtained in 1920–1921 at the Hopkins Marine Station of Leland Stanford University at Pacific Grove and at the Marine Biological Laboratory at Woods Hole. I wish to express here my appreciation of the hospitality extended to investigators at the Hopkins Marine Station and my thanks to the Director, Dr. W. K. Fisher, and to Dr. Gertrude Van Wagenen, for their assistance and encouragement. The photomicrographs accompanying this paper were taken for me by Dr. Doane, of the Department of Entomology of Stanford University. For the use of a research room at Woods Hole I am indebted to the Director, Dr. F. R. Lillie, and for suggestions and criticisms to Dr. O. C. Glaser.

II. ISO-AGGLUTINATION.

1. General.

Iso-agglutination is characterized by the rapid formation of dense spherical swarms of intensely active adherent spermatozoa and by the subsequent reversal of this process, Lillie (1921). The duration of the reaction varies with the concentration and freshness of the sperm suspension and of the egg-water, Lillie (1914, 1915), and is obtained only with motile spermatozoa, Loeb (1914). This reaction has been observed in a few marine animals and wherever it occurs indicates that we are dealing with ripe reproductive cells, species true. Its significance lies in its specificity, Lillie (1921).

Iso-agglutination has been reported by Lillie (1912, 1913) for *Arbacia punctulata* and for *Nereis;* by Glaser (1914) for *Asterias forbesii;* and by Just (1919) for *Echinarachnius.* Loeb (1914) described "cluster formation" in *Strongylocentrotus purpuratus* and in *S. franciscanus*. This, as Lillie (1921) later determined, is identical with iso-agglutination.

2. Material and Methods.

Material.—The animals used in this work represent two phyla, Echinodermata and Mollusca. Belonging to the first are *Stronglyocentrotus purpuratus, S. franciscanus, Arbacia punctulata, Asterias ochracea,* and *Lepasterias æqualis;* and to the second *Katharina tunicata, Ishnochiton magdalenensis, Mopalia muscosa, Cryptochiton,* and *Abalone.*

Methods.—Every precaution is taken to prevent contamination of the gametes with body fluids. With the three species of sea-urchins this consists of washing animals and dissecting instruments with tap water, cutting around the oral disc and removing all body contents except the gonads, and then washing the cavity thoroughly with sea-water. The animals are then placed on their aboral surfaces in Syracuse watch glasses and allowed to shed their gametes through the germinal pores. A second method, suggested by Dr. O. C. Glaser, consists of washing the animals in tap water, rubbing off the spines, and drying with a towel. They are then allowed to shed as in the first method. Practically dry gametes can be obtained by this method. In the case of the starfish and molluscs the same method of sterilization is employed. The rays of the starfish are removed and the gonads, stripped from each ray, are transferred to a finger bowl of filtered sea-water and thoroughly washed. In the molluscs the gonads lie just beneath a dorsal shell and can be reached in one of two ways. The first consists of removing the ventral muscle and viscera with the exception of the gonads, and the second of removing the shell. The exposed gonads can then be thoroughly washed with sea-water, removed with blunt forceps to Syracuse watch glasses or finger bowls, and ruptured. In this manner dry gametes can be procured.

One (1) per cent. suspensions of spermatozoa are made by adding to 99 drops of filtered sea-water 1 drop of dry sperm. Pipettes known to deliver the same number of drops per c.c. are used. All suspensions are used within ten minutes or discarded. Standard egg-water is prepared by allowing one volume of dry ripe eggs to secrete into two volumes of filtered sea-water for ten minutes. The egg-water is then separated from the eggs by filtration or centrifugation. The agglutination test is performed as follows: A drop of 1 per cent. sperm suspension placed between a slide and a cover glass supported by mm. glass rods spreads out into a thin film, and in it the spermatozoa may be studied microscopically. A fine-pointed capillary glass tube attached to rubber tubing is used for blowing drops of test solutions into the thin film of sperm suspension.

3. Iso-agglutination in Sea-urchins.

The results of my own experiments substantiate the statement of F. R. Lillie (1919, 1921) that egg secretions of *Arbacia, S. purpuratus,* and *S. franciscanus* produce three effects on species-true spermatozoa: *activation,* stimulation to increased motility followed later by a state of rest; *aggregation,* a tropistic phenomenon occurring only when there is a gradient from the egg-secretion to the spermatozoa; and *agglutination.*

Iso-agglutination Reaction.

An immediate and intense activation and the aggregation of the spermatozoa into a dense ring around the injected drop of egg-water is followed by the agglutination reaction. The ring rapidly becomes beaded in appearance and ultimately breaks up into small swarms. In each of the latter the spermatozoa are in such rapid motion that the entire swarm whirls about. Simultaneously similar smaller swarms form from the few spermatozoa trapped within the enclosed drop. For a brief period the swarms appear to rush together to form larger masses. The movements of the spermatozoa gradually slacken, and after a short interval, depending on the size of the swarms, a reversal occurs. They break up and in a short time the spermatozoa are dispersed, less active than originally.

In a special series of experiments I used spermatozoa of *Arbacia* from 25 per cent. and 50 per cent. suspensions which had stood at room temperature (21° C.) for from twelve to twenty-four hours, and were then aërated to remove the carbon dioxide, which in itself might affect the agglutination process. In 1 per cent. suspensions of such spermatozoa I noticed an intermediate stage in the process of agglutination. This intermediate stage occurs during reversal. It is characterized by a radial orientation of the active spermatozoa such that the swarm momentarily takes on the appearance of a three-dimensional pinwheel. At the center of the wheel is a "nucleus" of sperm heads with tails radiating outward, while the periphery of the wheel is composed of a dense zone of sperm heads with their tails radiating inward. In optical section it is as though a set of spokes originating at the hub of a wheel were dovetailed between another set radiating inward from the rim. These pinwheels gradually break up and eventually the spermatozoa are completely dispersed as in typical reversible agglutination. This pinwheel formation is not comparable to a secondary aggregation which often follows iso-agglutination. In such aggregation the masses formed are irregular in shape; and the spermatozoa composing them are not intensely active, not oriented, and are readily dispersed by shaking.

4. Iso-agglutination in *Katharina tunicata.*

The spermatozoa of *K. tunicata* are inactive or but slightly active in sea-water. They can be roused to intense activity by foreign blood or foreign tissue extracts and exhibit a spiral method of locomotion similar to that described for other types of spermatozoa. In contact with surfaces they move anti-clockwise. This is probably due to their structure. (See Fig. 1.) As judged by their activation by various substances the spermatozoa of this species were ripe as early as April 9. Egg-water tested April 15, May 2, and May 16 failed to produce even an activation of spermatozoa. Inseminated eggs did not develop, indicating that the eggs were not ripe. On May 27 secretions from ripe eggs of females caught on the same day caused intense activation, aggregation, and a peculiar type of agglutination. The latter is comparable to the intermediate stage in the iso-agglutination reaction obtained with stale spermatozoa of *Arbacia.*

Following activation and aggregation of the spermatozoa into a dense ring, the latter rapidly break up into three dimensional pinwheels instead of into the whirling swarms characteristic of the iso-agglutination in sea-urchins and in *Nereis.* Within the injected drop of egg-water where the spermatozoa are less concentrated it is possible to observe the process of formation of these pinwheels. A few spermatozoa first stick together in a group without any apparent orientation and without enough motility to produce whirling of the group. The three-dimensional pinwheels form instantly, including these clumps within the wheel. A fusion of pinwheels ensues for a short period. A complete reversal then occurs in some, whereas other pinwheels remain permanent. A protocol of one experiment will illustrate the time relations of the phenomenon.

Exp. 616.—Material: Doubly filtered standard egg-water of *K. tunicata;* fresh 1 per cent. sperm suspension of *K. tunicata.*
5–27/21.

3.15. Sperm suspension in sea-water—spermatozoa slightly active.
3.15. Inject a drop of egg-water—
    Immediate intense activation.
    Immediate ring formation.
3.16. Formation of small clumps of spermatozoa.
3.165. Formation of three-dimensional pinwheels.
3.17. Fusion of three-dimensional pinwheels.
3.19. Complete reversal of some pinwheels.
3.25. Decrease in activity; many permanent pinwheels remain.
3.40. No change.
5.00. No change. Spermatozoa still more active than originally.

On May 28 I obtained with animals brought in on the preceding day decided activation and ring formation, but no agglutination. In previous tests on spermatozoa of this species I had discovered that in order to obtain satisfactory results animals must be used on the day on which they are obtained. During June and July of 1921 and 1922 additional experiments were conducted for me by Dr. Van Wagenen. She reports results similar to those recorded in Exp. 616. However, complete reversal of agglutination was obtained with both standard egg-water and with the latter diluted ten times. Dilutions of $\frac{1}{100}$ and $\frac{1}{500}$ produced activation but no agglutination, thus indicating a rapid loss of agglutinating power with dilution.

5. Iso-agglutination in Other Echinoderms and Molluscs.

Iso-agglutination tests were made upon the spermatozoa of certain other Echinoderms and Molluscs: *Asterias ochracea,* *Lepasterias æqualis, Asterias forbesii; Ishnochiton magdalenensis, Mopalia muscosa, Cryptochiton, Abalone,* and *Cumingia.* Both sperm and ova were ripe.

Activation occurred in every case, but no agglutination comparable to that in sea-urchins, *Nereis* or *K. tunicata.* In *Asterias forbesii, Asterias ochracea,* and *Cumingia* irregular clumps form consisting of a few spermatozoa. These, however, are irregularly dispersed and hence do not correspond to those which appear preceding typical iso-agglutination.

Table I.

The Effects of Egg-Water on Spermatozoa of the Same Species.

	Spermatozoa.				
	Arbacia punctulata *Strongylocentrotus purpuratus* *Strongylocentrotus franciscanus* *Nereis limbata*	*Arbacia punctulata* (stale spermatozoa)	*Katharina tunicata*	*Asterias forbesii* *Asterias ochracea* *Cumingia*	*Lepasterias aequalis* *Ishnochiton magdalenensis* *Mopalia muscosa* *Cryptochiton* *Abalone*
Motility in sea-water	+	+	−	−	−
Effects of egg-water:					
Activation	+	+	+	+	+
Aggregation	+	+	+	−	−
Clumping of 3–10	+	+	+	+	−
Dispersal uniform	+	+	+	−	
Dispersal irregular	−	−	−	+	
Agglutination-swarms	+	+	−	−	−
Agglutination-pinwheels	−	+	+	−	−
Reversal of agglutination:					
Partial	−	−	−		
Complete	+	+	+		
Decrease in motility	+	+	+	+	+
Strand formation	−	−	−	−	−
Cytolysis	−	−	−	−	−

6. Discussion.

The variation in character of the iso-agglutination reaction may be due in part to the degree of motility of the spermatozoa involved. Swarming is obtained with spermatozoa highly motile in sea-water; the pinwheel type with spermatozoa inactive in sea-water (*K. tunicata*), or with spermatozoa rendered less active in sea-water by staling (*Arbacia*). The fact that spermatozoa of both *Arbacia* and of *K. tunicata* move anti-clockwise when in contact with other objects may account for the shape of the pinwheels.

Of the ten species tested in which the spermatozoa are inactive in sea-water, iso-agglutination occurred in but one species, *Katharina tunicata.*

III. HETERO-AGGLUTINATION.

1. General.

Two distinct types of hetero-agglutination were obtained in this investigation, one with sea-urchins and one with *K. tunicata.* Hence their characteristics will be considered separately and compared with other accounts of the phenomenon.

Hetero-agglutination has been reported by Lillie (1913) between *Arbacia* egg-water or blood and *Nereis* spermatozoa; by Glaser (1914) between *Arbacia* egg-water and *Asterias* spermatozoa, and the reciprocal relationship between *Asterias* egg-water and *Arbacia* spermatozoa; by Just (1919) between *Arbacia* egg-water and *Echinarachnius* spermatozoa; and by Loeb (1914) between *S. purpuratus* egg-water and *S. franciscanus* spermatozoa. Reciprocal hetero-agglutination has been reported but once, as indicated above.

Evidence is given here of hetero-agglutination of spermatozoa of *K. tunicata* by solutions of cytolyzed spermatozoa of *S. purpuratus;* also by the blood of either *S. purpuratus* or *S. franciscanus.* The reciprocal relationship is also reported: hetero-agglutination of spermatozoa of *S. purpuratus* and of *S. franciscanus* by the blood of *K. tunicata.*

2. Material and Methods.

The material and methods include those employed in iso-agglutination experiments. In addition, blood was collected and filtered and solutions of cytolyzed spermatozoa of *S. purpuratus* and *S. franciscanus* were prepared in the following manner:

A 5 per cent. suspension of spermatozoa in glass distilled water was allowed to stand at room temperature (15° C.) for one hour, shaken at frequent intervals, and filtered (through Whatman filter paper, No. 2 and No. 50) three times.[1] In order to make the solution equal in specific gravity and hydrogen-ion concentration to sea-water, concentrated sea-water and $N/100$ NaOH were added. Controls were arranged by adding to glass distilled water concentrated sea-water and $N/100$ HCl. In making the corrections I used a standard hydrometer, and a set of standards prepared by Hynson, Westcott, and Dunning for determining the hydrogen-ion concentration of sea-water.

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[1] Solutions made from spermatozoa which were allowed to cytolyze in dis-

3. Hetero-agglutination of Spermatozoa of *Katharina tunicata.*

A. Effect of Solutions of Cytolyzed Spermatozoa of S. purpuratus.

The spermatozoa of *K. tunicata,* inactive in sea-water, are intensely activated and agglutinated by solutions of cytolyzed spermatozoa of *S. purpuratus.* The reaction resembles iso-agglutination in this species in the formation of three-dimensional pinwheels and differs from it only in the irreversibility of the hetero-agglutination (Plate I.). In forty-five experiments, in which two different test solutions were employed over a period of 34 days, similar results were obtained. A single experiment will illustrate the characteristics of the reaction.

Exp. 511.—Material: *K. tunicata* spermatozoa; solution of cytolyzed spermatozoa of *S. purpuratus.*

3–21/21—2.23 P.M.

Time.	Activation.	Agglutination.	Pinwheel Formation.	Reversal.
2.23	Intense	0	0	–
2.24	Intense	0	0	–
2.245	Intense	Small clumps	Few and small	–
2.25	Intense	0	Fusion of small clusters	0
2.45	Intense	0	Large and numerous	0
3.05	Very active	0	Large and numerous	0
3.45	Slightly active	0	Large and numerous	0
5 00	Slightly active	0	Large and numerous	0

As indicated above, the formation of pinwheels occurs after a latent period of 1½ minutes in this experiment. In other experiments the latent period was often shorter, but in no case less than 20 seconds.

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tilled water from 3–5 hours caused activation but no agglutination. This may be due to an unstable property of the hetero-agglutinating substance in distilled water.

Effect of Dilution on Hetero-agglutinating Power of Solutions.—Lillie (1915) found in the case of hetero-agglutinins in *Arbacia* egg-water a disproportional loss of agglutinating power with dilution, and certain preliminary experiments with one half and one fourth dilutions of solutions of cytolyzed spermatozoa of *S. purpuratus* indicated a similar loss. The results obtained with a series of greater dilutions are indicated in the following table:

TABLE II.

THE EFFECT OF DILUTION ON STRENGTH OF HETERO-AGGLUTINATION OF SOLUTIONS OF CYTOLYZED SPERMATOZOA OF *S. purpuratus*.

	Activation.			Pinwheels.		
	Dilution.	Time.	Degree.	Time.	Number.	Size.
1.....	0	Immediate	Intense	After 20″	Many	Large
2.....	1/10	Immediate	Intense	After 4′	Many	Small
3.....	1/20	Immediate	Slight	After 2.5′	Few	Small
4.....	1/30	Immediate	Slight	After 3′	Few	Small
5.....	1/40	Immediate	Slight	After 5′	Few	Small
6.....	1/50	Immediate	Slight	After 3′	Few	Small
7.....	1/60	Immediate	Slight	After 5′	Rare	Minute

In the above summary the decrease in agglutinating power appeared to be associated with a decrease in activating power of the diluted solution of cytolyzed spermatozoa. One might predict that an activating substance added to cytolyzed sperm solutions would prevent the loss of agglutinating power. This actually proved to be true, for upon the addition of an activating body a dilution of 1/60 produced as intense and immediate activation and pinwheel formation as the undiluted solution.[1]

B. *Effect of the Blood of S. purpuratus and S. franciscanus.*

Filtered blood of both male and female *S. purpuratus* and *S. franciscanus* causes activation and hetero-agglutination of spermatozoa of *K. tunicata* exactly like that produced by solutions of cytolyzed spermatozoa of *S. purpuratus.* The blood must, however, be taken from the animals on the day on which they are taken from their habitat. Otherwise it will produce activation, but no agglutination. This is comparable to the deterioration of spermatozoa in animals kept in the laboratory. Blood taken from fresh animals, however, retains its hetero-agglutinating power for at least three days.

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[1] The substance in question will be discussed in a subsequent paper.

With blood, as with solutions of cytolyzed spermatozoa, there is a disproportionate loss of agglutinating power with dilution.

C. *Effect of the Egg-Water of S. purpuratus and of S. franciscanus.*

The egg-water of ripe ova of *S. purpuratus* and of *S. franciscanus* failed repeatedly to produce either activation or hetero-agglutination of the spermatozoa of *K. tunicata.* Considering that the latter can be intensely activated and agglutinated by blood and by solutions of cytolyzed spermatozoa of these two species of sea-urchins, and in view of the striking resemblance of iso-agglutination and hetero-agglutination in *K. tunicata,* it is especially significant that the egg-water of both species of *Strongylocentrotus* fails to produce hetero-agglutination of *K. tunicata* spermatozoa. This constitutes further evidence of the specificity of the iso-agglutinating substance present in egg-waters.

D. *Changes in Spermatozoa Produced by Hetero-agglutinating Substances.*

In solutions which produce hetero-agglutination the heads of the spermatozoa of *K. tunicata* become swollen at the base, as indicated in Fig. 1.

A comparison of the dimensions of spermatozoa in sea-water and in hetero-agglutinating solutions will illustrate this:

Spermatozoa.	Sea-water.	Hetero-agglutinating Solution.
Head length	$10\,\mu$	$10\,\mu$
Head width at base	$2\,\mu$	2.25 to $3.35\,\mu$

E. *Discussion.*

Hetero-agglutinating substances have previously been demonstrated in blood and in egg-water. Just and Lillie have suggested that in the case of *Arbacia* the hetero-active substance is a constituent of the blood, and that egg-water used was contaminated with blood. The hetero-active substance in the egg-water of *S. purpuratus* for *S. franciscanus* is, however, not a normal constituent of the blood of *S. purpuratus,* Lillie, 1921. It is improbable that contamination with blood can account for the strong hetero-agglutinating power of the solutions of cytolyzed sperm made from 5 per cent. suspensions of spermatozoa. Such a dilution of whole blood would have slight hetero-agglutinating properties.

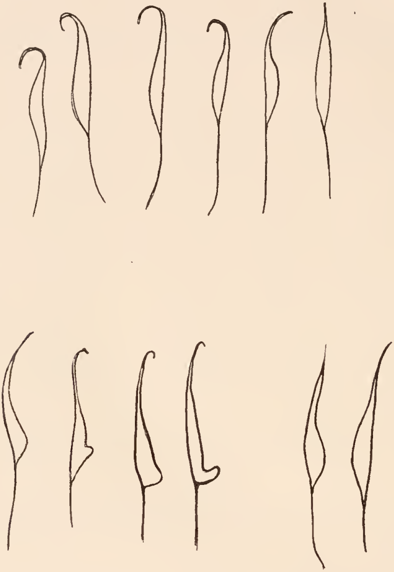

Fig. 1. Spermatozoa of *Katharina tunicata.*

a. In sea-water—normal. Mag. x 5,000 (approx.).

b. In solution of cytolyzed spermatozoa of *S. purpuratus.* The heads are swollen at the base. Mag. x 5,000.

It is possible that the products of cytolysis of eggs or of tissue cells of these two species of sea-urchins would also cause hetero-agglutination of spermatozoa of *K. tunicata.*

4. Hetero-agglutination of Spermatozoa of *S. purpuratus* and of *S. franciscanus* by the Blood of *Katharina tunicata.*

Since spermatozoa of *K. tunicata* were agglutinated by the blood and by solutions of cytolyzed spermatozoa of *S. purpuratus* and by the blood of *S. franciscanus,* it seemed possible that the relationship might be reciprocal. This proved to be the case, but the type of hetero-agglutination obtained resembles that described by Lillie as "mass coagulation." A single protocol will illustrate the nature of the reaction.

Exp. 585.—Blood of *K. tunicata;* spermatozoa of *S. purpuratus.* 5/4/21—2.15 P.M.

Time.	Activation.	Ring Aggregate.	Formation of		Strands.	Reversal.
			Swarms	Pinwheel.		
2.16.....	Intense	0	0	0	Few	–
2.165....	Intense	0	0	0	Many and united	0
2.25.....	Slight	0	0	0	Many and united	0

As indicated, there is no ring formation due to aggregation. The spermatozoa rapidly form strands which adhere to one another, and lose their motility. This hetero-agglutination is completely irreversible and decidedly toxic. It is of interest that the blood of *K. tunicata* which causes hetero-agglutination of spermatozoa of *S. purpuratus* and of *S. franciscanus* also causes membrane formation in the eggs of these two species. If allowed to act too long, it will induce cytolysis. A short treatment followed by a brief exposure to hypertonic sea-water will, however, lead to parthenogenetic development of the ova of *S. franciscanus,* Sampson (unpublished).

5. Hetero-agglutination in Other Echinoderms and Molluscs.

Tests were made with the spermatozoa of other Echinoderms and Molluscs to demonstrate hetero-agglutination. The results of these tests indicate two distinct types of hetero-agglutination: (*A*) the toxic "mass coagulation," described by Lillie for *Nereis* spermatozoa; (*B*) the pinwheel type, described in this report for

K. tunicata; and (*C*) a questionable third type "clumping," described by Glaser for *Arbacia.* (The "clumps" are irregularly distributed and resemble aggregation rather than hetero-agglutination.) A summary of the results is given in Tables III. and IV. These tables also include results first reported by Glaser, Just, Lillie, and Loeb, as indicated by the initials in brackets.

TABLE III.

HETERO-AGGLUTINATION OF SPERMATOZOA OF CERTAIN MARINE ANIMALS (WOODS HOLE, MASS.).

Test Solutions.	Spermatozoa.					
	Arbacia punctulata.	*Echinarachnius parma.*	*Nereis limbata.*	*Asterias forbesii.*	*Cumingia tellinoides.*	*Chiton apiculata.*
Arbacia punctulata						
Egg-water.............		+ *A*(J)	+ *A*(Li)	+ *C*(G)	+ *C*	–
Blood.................		+ *A*(J)	+ *A*(Li)			–
Sperm suspension.......			+ *A*(Li)			
Echinarachnius parma						
Egg-water.............	– (J)					
Blood.................	– (J)					
Nereis limbata						
Egg-water.............	– (Li)					
Blood.................	– (Li)					
Sperm suspension.......	– (Li)					
Asterias forbesii						
Egg-water.............	+ *C*(G)					
Cumingia tellinoides						
Egg-water.............	+ *C*					
Chiton apiculata						
Egg-water.............	+ *A*					
Blood.................						

The letters *A* and *C* refer respectively to the strand formation (Lillie, 1913) and clumping (Glaser, 1914) described in reports on hetero-agglutination.

IV. SUMMARY.

1. Iso-agglutination occurs in the black chiton, *Katharina tunicata.* This is the first report of unmistakable iso-agglutination of spermatozoa of a mollusc; also of iso-agglutination of spermatozoa which are inactive in sea-water.

2. The iso-agglutinated masses of spermatozoa of *K. tunicata* differ from those of sea-urchins and of *Nereis.* In the former the agglutinating masses resemble three-dimensional pinwheels.

TABLE IV.

HETERO-AGGLUTINATION OF SPERMATOZOA OF CERTAIN MARINE ANIMALS (PACIFIC GROVE, CALIF.).

Test Solutions.	Spermatozoa.								
	Strongylocentrotus purpuratus.	*Strongylocentrotus franciscanus.*	*Asterina.*	*Asterias ochracea.*	*Katharina tunicata.*	*Ishnochiton magdalenensis.*	*Mopalia muscosa.*	*Cryptochiton stelleri.*	*Abalone.*
Strongylocentrotus purpuratus									
Egg-water..........		+(Lo)	–(Lo)	–(Lo)	–	–	+*C*	–	–
Blood..............	–(Li)	–(Li)		–(Lo)	+*B*	+*A*	–	–	–
Cytolyzed sperm.....	–	–		–	+*B*	–	–	–	–
Strongylocentrotus franciscanus									
Egg-water..........	–(Lo)		–(Lo)	–(Lo)	–	–	–	–	–
Blood..............	–(Lo)	–(Lo)		–(Lo)	+*B*	+*A*	–	–	–
Asterina									
Egg-water..........	–(Lo)	–(Lo)							
Asterias ochracea									
Egg-water..........	–(Lo)	–(Lo)							
Blood..............	–(Lo)	–(Lo)		–(Lo)					
Katharina tunicata									
Egg-water..........	+*A*	+*A*		–					
Blood..............	+*A*	+*A*		+*C*	–				
Ishnochiton magdalenensis									
Egg-water..........									
Blood..............	+*A*	+*A*				–			
Mopalia muscosa									
Egg-water..........									
Blood..............							–		
Cryptochiton stelleri									
Egg-water..........									
Blood..............								–	
Abalone									
Egg-water..........									
Blood..............									–

The letters used refer to types of hetero-agglutination (*A*) to strand formation; (*B*) to pinwheel formation; and (*C*) to clumping. The latter may be considered as aggregation rather than as a type of hetero-agglutination. See text.

In these the spermatozoa are so oriented that the center consists of a nucleus of sperm heads with tails radiating outward, and the periphery is composed of a dense zone of sperm heads with tails radiating inward.

In sea-urchins and in *Nereis* the iso-agglutinating masses resemble whirling swarms in which the spermatozoa either are not oriented or are oriented with their heads forming the central nucleus of the swarm. However, a pinwheel type of iso-agglutination can be obtained with stale *Arbacia* spermatozoa. The motility of the latter is decidedly subnormal. It is possible that the variation in shape of agglutinated masses may be correlated with the degree of motility of the spermatozoa which are involved.

3. Unmistakable iso-agglutination can not be detected in any of the following: *Asterias ochracea, Asterina, Asterias forbesii, Lepasterias æqualis, Cumingia, Ishnochiton magdalenensis, Mopalia muscosa, Cryptochiton,* and *Abalone.*

4. Two types of hetero-agglutination are here reported: toxic "mass coagulation," similar to that described by Lillie (1913) for *Nereis* and by Just (1919) for *Echinarachnius,* and a pinwheel type in *Katharina tunicata,* which bears a startling resemblance to iso-agglutination in the same species.

5. Hetero-agglutination occurs between spermatozoa of *K. tunicata* and solutions of cytolyzed spermatozoa or of blood of either *S. purpuratus* or of *S. franciscanus.* The reciprocal also occurs: hetero-agglutination of spermatozoa of either *S. purpuratus* or of *S. franciscanus* by the blood of *K. tunicata.*

6. Hetero-agglutination of spermatozoa of *Ishnochiton magdalenensis* may be produced by the blood of either *S. purpuratus* or of *S. franciscanus.* The reciprocal may also be produced: hetero-agglutination of spermatozoa of either *S. purpuratus* or of *S. franciscanus* by blood of *Ishnochiton magdalenensis.*

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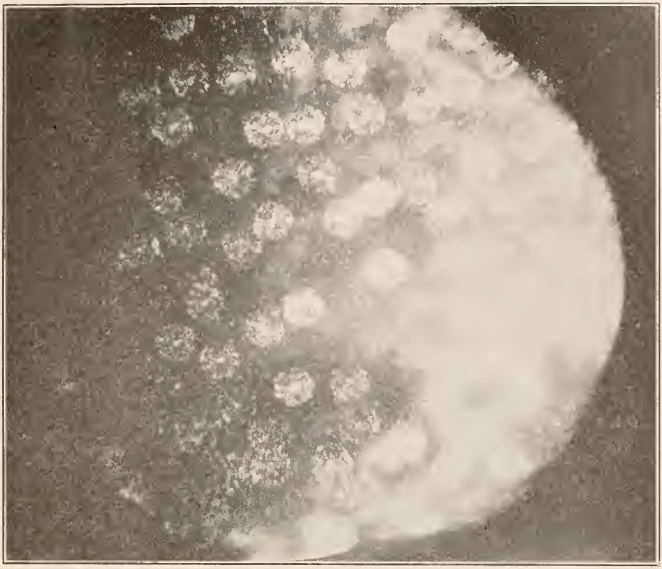
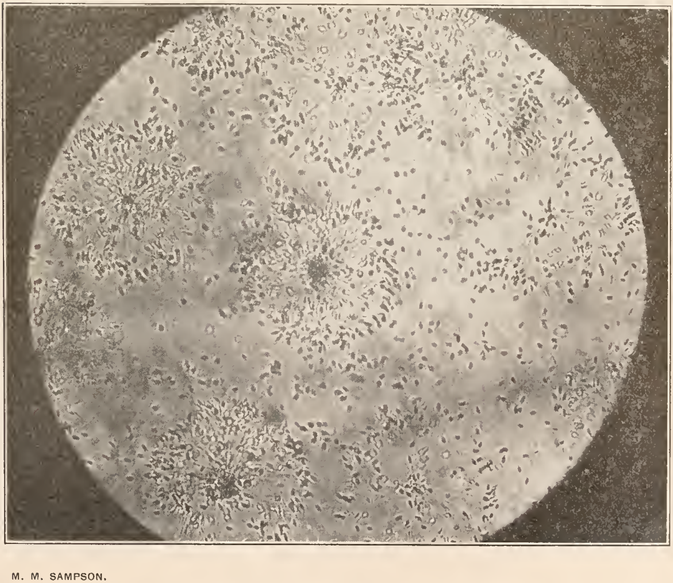
PLATE I.

Photomicrographs of Hetero-agglutination of Spermatozoa of *Katharina tunicata* in a solution of cytolyzed spermatozoa of *Strongylocentrotus purpuratus.*

The spermatozoa form three-dimensional pin-wheels.

Magnification × 60.
Magnification × 260.





M. M. SAMPSON.

ON THE PHYSIOLOGICAL PROPERTIES OF THE GONADS AS CONTROLLERS OF SOMATIC AND PSYCHICAL CHARACTERISTICS: V. THE EFFECTS OF GONADECTOMY IN THE GUINEA PIG, ON GROWTH, BONE LENGTHS, AND WEIGHT OF ORGANS OF INTERNAL SECRETION.

CARL RICHARD MOORE,

THE UNIVERSITY OF CHICAGO. HULL ZOÖLOGICAL LABORATORIES.

To be able to interpret correctly the changes in weight of an animal incident to experimental modification of the sex-gland conditions, such as total castration or spaying followed by sex-gland transplantations, it is necessary to know the relationship of the weight of the normal to the totally castrated and totally spayed animal. Certain investigators have employed relative weights of the rat and guinea pig as indications of modification of the sexual conditions (see Steinach and Holznecht), and certainly the reactions of the guinea pig, in this respect, are not sufficiently understood to justify such conclusions as have been announced.

Steinach takes for granted that, inasmuch as the male of both rats and guinea pigs is usually heavier than the female, the testis is responsible for an internal secretion liberated by the interstitial cells that promotes growth and otherwise leads to the production of maleness, whereas the ovary is responsible for a secretion that acts to retard growth. Acting upon this assumption, he utilizes the weight of an animal at practically any age to compare with a brother or sister animal to show the differential effects of sex-gland grafts.

In 1919 the writer called attention to the work of Stotsenburg ('09, '13) on the rat wherein the growth curve was shown to be independent of the testis, but the growth curve of females was reduced by the presence of the ovary—*i.e.,* totally spayed female growth curves were from 17 per cent. to 30 per cent. higher than that of their normal sisters. It is to be understood, therefore, for the rat, that relative weight increase in a spayed female bearing a testis graft is due to the removal of the retarding influence of the ovary and not to an influence of the testis graft as claimed by Steinach. Sufficient data were presented in the second paper of this series[1] to show that Steinach's femininized and masculinized rats can not be so adjudged on the basis of differences in weight.

Since Steinach has applied the same principles to the guinea pig to detect changes in its sexual condition after operative procedures, the writer in 1921 criticized again such indiscriminate uses of weight records and mentioned an experiment under way to determine the fundamental reactions in weight upon removal of the gonads. The present paper embodies the results obtained from this investigation.

The growth of a series of guinea pigs, consisting of normal males and females, as well as totally castrated males and totally spayed females, was followed from birth to maturity (for one year). And in addition to determining the growth curves for the four classes of animals it appeared not only desirable to examine the glands of internal secretion for possible changes correlated with sex glands, but to examine as well the effect of castration and spaying on the growth of the long bones of the leg.

II. Material and Methods.

The animals for this experiment were selected from young born from our own laboratory stock at a time when the stock was able to supply a quantity within a relatively short period of time. Comparisons in weight were then made at approximately the same time of the year, as all the animals of this experiment were born between April 20 and August 27, 1920. The forty-six animals with which the experiment was begun were grouped in the following manner: 12 normal males, 11 castrated males, 12 normal females, and 11 spayed females. These were so caged as to be recognizable at any moment, and in such a manner that pregnancy was eliminated in the normal females in all but two cases.[2]

The animals were kept in the same room, in ordinary wire cages

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[1] Moore, '19.

[2] Minot, 1891, has shown that pregnancy does not cause a permanent change in the weight of a female animal; a correction for the weight of the unborn young was made in these two cases of pregnancy.

16 x 16 x 30 inches, were fed daily with carrots and hay, and usually all cages were supplied with grain (corn, oats) twice each week. Each animal was weighed on each thirtieth day from birth to the end of one year (360 days). As each weighing was made by the writer, an absence of three weeks caused one weighing of some animals to be omitted.

To avoid differences due to the operation castration was accomplished by opening the peritoneal cavity of the young male by a mid-ventral incision; the spermatic cord was tied off considerably above the testis, and by cutting the cord below the ligature the testis was removed intact. Spaying was accomplished by means of two dorsolateral lumbar incisions (one on either side) tieing, or clamping for a short time, the ovarian blood vessels, and removal of the ovary with or without a considerable amount of the oviduct. Post-mortem examination showed that in every case the entire ovary had been removed. Each animal was castrated or spayed before the thirtieth day and in the majority it was done before the age of fifteen days.

As the experiment approached its termination each animal was killed with chloroform immediately after weighing (on the 360th day) and the following data recorded: total body weight (before killing), total body length, weight of the two thyroids, hypophysis (pituitary body), the two adrenals, spleen, sex glands where present, and the lengths of the femur, tibia, and fibula.

In weighing the glands, except the hypophysis, all were rapidly removed from the animal, cleaned of superfluous connective tissue, and placed together in a ground-glass covered container, weights being obtained by difference as each gland was removed to fixation bottles. The weights were obtained as rapidly as possible, hence the glands contained whatever blood or other fluids they possessed at the moment of removal.

In handling the pituitary, the cranial cavity was rapidly opened, cranial nerves severed as the brain was lifted off the floor of the cranial cavity, and the gland very carefully dissected from under the membranes and immediately dropped into a weighing tube containing the desired fixing fluids; the weight of the container and fluid had been determined immediately before the animal was killed. In all cases the hypophysis was in the killing fluid and weighed within thirty minutes after the administration of chloroform. In each animal thyroids, adrenals, and hypophysis were preserved in Bouin's fluid or formol-Zenker's solution for subsequent histological study.

In bone measurement the two legs of each animal were stripped of muscle and boiled in a weak alkali solution for a short time, after which they were cleaned of all muscle and ligaments. They were then measured by means of graduated calipers (grad. to .01 cm.) and the length of the two corresponding bones averaged. Second measurements were made at a later date by Mr. N. F. Fisher, who was unacquainted with my measurements, and the two sets compared; in case of differences a third measurement was taken.

III. Growth Curves.

The weights of each animal of the experiment on each thirty days is given in Table I., and the growth curves for the four groups are shown in Fig. 1. These curves were constructed from an average of the weights of all animals in each of the four groups for each weighing period—*i.e.,* 30, 60, 90 days, etc.

Table I.

Showing Individual Weights of Each Animal up to 360 Days and the Averages for Each Group as a Whole at Each Weighing Period.

Animal.	30 da.	60 da.	90 da.	120 da.	150 da.	180 da.	210 da.	240 da.	270 da.	300 da.	330 da.	360 da.
Normal Males												
X1......	172	325	500	—	680	700	760	810	865	870	930	980
X2......	205	305	440	640	—	740	770	825	830	885	895	835
X3......	180	330	495	—	630	670	720	765	815	855	807	870
X4......	162	270	450	625	—	730	805	855	930	980	980	967
X5......	120	200	330	—	440	475	520	520	580	600	450	575
X6......	210	375	580	—	680	715	770	810	825	890	970	950
X7......	255	405	505	580	520	650	705	725	790	810	775	810
X8......	340	540	—	725	830	930	980	1,065	920	1,040	1,115	1,120
X9......	245	455	—	630	700	755	720	665	670	685	660	650
X10.....	155	295	—	425	490	500	535	555	585	605	650	550
X16.....	280	—	465	540	595	620	660	725	725	785	860	885
X22.....	—	510	560	670	810	920	1,040	1,080	1,150	1,220	1,195	1,280
Average..	211	364	480	604	637	700	749	783	807	852	857	872

Castrated Males

X11.....	135	245	—	365	410	490	510	540	565	600	600	555
X12.....	—	450	—	560	615	650	750	dead	—	—	—	—
X13.....	—	445	—	545	585	575	605	700	755	795	810	850
X14.....	165	285	400	465	535	615	690	735	800	835	870	940
X15.....	370	515	605	635	700	740	765	825	855	dead	—	—
X17.....	280	—	470	525	595	635	650	720	748	dead	—	—
X18.....	240	—	410	440	510	565	640	685	715	750	795	830
X19.....	270	—	450	500	580	640	725	770	dead	—	—	—
X20.....	—	365	415	415	510	555	530	585	580	620	655	695
X21.....	—	360	455	545	570	650	dead	—	—	—	—	—
X23.....	—	415	470	605	735	740	845	907	925	940	1,015	995
Average..	243	385	459	509	576	623	671	718	742	756	791	810

Animal.	30 da.	60 da.	90 da.	120 da.	150 da.	180 da.	210 da.	240 da.	270 da.	300 da.	330 da.	360 da.
Normal Females												
Y1......	175	305	460	—	640	670	740	700	700	700	730	807
Y2......	285	370	510	645	—	760	800	875	900	945	980	880
Y3......	197	265	475	620	—	770	780	815	855	865	855	925
Y4......	170	210	390	515	—	640	675	750	770	805	875	875
Y5......	130	245	360	—	590	dead	—	—	—	—	—	—
Y7......	180	390	615	—	670	720	760	dead	—	—	—	—
Y8......	222	380	—	590	640	665	740	785	790	815	870	900
Y9......	215	360	—	550	590	645	705	590	670	760	815	800
Y10.....	250	390	—	580	620	685	720	780	835	850	830	890
Y19.....	—	390	435	520	625	640	685	745	795	855	900	975
Y20.....	—	335	370	435	510	540	585	620	655	700	730	785
Average..	202	330	452	557	610	673	719	740	774	810	843	871
Spayed Females												
Y11.....	240	390	—	605	660	735	780	840	870	905	dead	—
Y12.....	370	530	—	680	735	740	745	760	835	917	930	995
Y14.....	163	280	375	455	515	593	645	700	765	815	850	915
Y15.....	300	400	485	550	590	580	645	720	700	740	715	700
Y16.....	262	—	440	490	565	620	630	655	680	740	810	850
Y17.....	—	330	365	435	510	560	575	625	650	695	755	800
Y18.....	—	345	390	460	520	570	595	630	655	695	755	800
Y21.....	—	395	510	590	630	695	dead	—	—	—	—	—
Y22.....	—	415	470	575	680	735	795	845	835	890	920	925
Y23.....	—	470	490	520	625	700	690	803	850	935	1,020	1,045
Average..	267	395	440	536	603	652	677	730	760	815	844	879

Examining the growth curves one sees that each group as a whole ascends in an unbroken curve from the first weighing to the last one made on the 360th day. A few animals of each series showed individual temporary loss in weight, but as a group the increases from month to month were continuous.

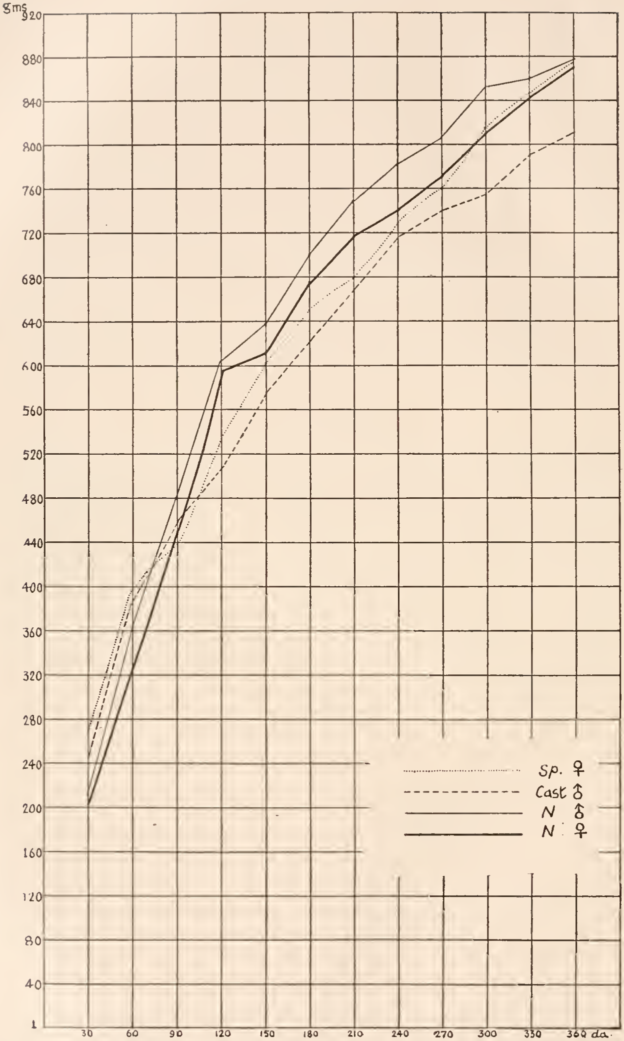

FIG. 1. Growth curves for normal males (N♂), normal females (N♀), totally castrated males (*Cast* ♂), and totally spayed female (*Sp* ♀), guinea pigs for one year from birth. The curves were constructed from the average weight of each group, on 30, 60, 90 days, etc., as given in Table I.

In reference to growth curves, as well as to all other elements of the investigation, three particular points have been kept in mind: (1) the possible demonstration of sexual differences, (2) the effects conditioned by total castration, and (3) the effects conditioned by total spaying.

Sexual Differences.—Comparing the growth curves of the normal animals, male with female (Fig. 1), one sees that the males are constantly the heavier up to the end of the first year of growth, when the average weight of the two groups is the same. This agrees with the findings of Minot ('91), who demonstrated that the male guinea pigs were heavier than the females (as a group) up to about the end of the first year, after which the female growth curve crossed that of the males, and from this time on the females were from 1 per cent. to 7 per cent. heavier than the males.

Total Castration.—From a glance at the growth curves in Fig. 1, or reference to the average weights from both groups of animals as seen in Table I., it becomes apparent that castrated males are lighter in weight than normal males at all ages from ninety days to the end of one year. In other words, castration causes a relative loss in weight in the guinea pig when compared with the normal male.

Total Spaying.—Spayed females also exhibit a relative loss in weight when compared with the normal females. The young spayed females at the beginning of the experiment were slightly heavier than the normal females, due merely to chance variation in weight in the selection of the animals, but at the age of ninety days the normal females are slightly heavier and continue so throughout the experiment until the 360th day, when the spayed females averaged approximately 1 per cent. heavier than the normal females. One is forced to conclude from this data that spaying results, for a certain period, in a decreased body weight (compared with the normal females), but that this loss is made good at a later time.

Discussion.—The particular point in mind for solution was whether the weight of an animal could be depended upon to offer an index of its sexual condition in case of sex-gland transplantations. As mentioned above, Steinach has placed considerable emphasis on the differences in weight after castration and spaying and subsequent transplantations of sex glands of the opposite nature. To mention specific data, Steinach and Holzknecht ('16, pp. 493 and 495) publish the following weights: (*a*) normal male, 980 grams; normal female, 808 grams; femininized males, 516 grams; and (*b*) normal female, 845 grams; normal male, 1,002 grams; masculinized female, 1,200 grams. Since the above writers believe that an ovary graft femininizes an animal, the data of (*a*) theoretically shows that the ovary graft has so functioned as to reduce the weight of the male to such an extent that it is 47 per cent. lower in weight than had it continued a normal male, and is indeed 36 per cent. below the weight of a normal female; thus the ovarian grafted male is more female than the normal female herself. Also, since a testis graft masculinizes an animal, the data of (*b*) indicates to them that the female, as a result of the testis graft, has gained 42 per cent. in weight above what it would have been had it remained a normal female, and to have increased in weight 19 per cent. above the normal male; thus, here also, the animal is considered much more a male, as an after effect of the testis graft, than the normal male.

Instead of showing that the ovary has a decided effect in lowering body weight, my own results on the guinea pig show that females deprived of the ovaries are lighter in weight up to the end of the first year than those possessing ovaries. While it is true the normal males are heavier than castrated males (indicating, possibly, that the testis promotes growth), nevertheless the averages of the two groups throughout the year give a difference of but 7.6 per cent. in favor of the normal males, as against the tremendous differences in the case of Steinach and Holzknecht. Comparisons of animals of the same litter do not afford more convincing arguments in favor of weight comparisons. One example being sufficient, attention is directed in Table I. to females Y19 and Y20. These two animals of the same litter (sisters) were kept in the same cage throughout their entire life and hence possessed equal chances of growth; each suffered no reversals, but continued to increase consistently for the 360 days, yet Y19 maintained a weight from 15 to 20 per cent. above Y20 throughout the entire period of observation. Let us suppose that Y19 had been spayed and received a testis graft at an early age. Comparisons would have shown that the animal had increased 20 per cent. in weight, as a result of the experiment, even had the testis not grown.

Without further discussion of the above point, it should be clearly demonstrated to the reader that such a comparison of weights of two or three animals, chosen at random, is absolutely unreliable as evidence of their sexual nature. As the writer has pointed out previously, the same criticism applies to rats. Since the fallacy of such comparisons can not but be realized, it follows that as evidence of masculinization and femininization such data should be ruled out when considering the value of the various types of data presented to support this idea.

Turning to the literature for actual data supporting the well-established idea that castration or spaying causes an overgrowth in body weight in animals (particularly the former), one realizes that there are indeed few experiments adequately controlled that give results supporting this idea.

Stotsenburg is apparently the first to carry out experiments on mammals determining the effects of gonadectomy wherein a sufficient number of animals were utilized to warrant definite conclusions. Reference was made above to these results. He found that the growth curves of normal and castrated male rats were very similar, and that castration in the rat did not result in a relative increase in weight from birth to maturity. In spayed females, however, there was a decided relative increase in weight as compared with normal females; the increase amounted to from 17 per cent. to 30 per cent. above the normal.

Hatai ('13, '15) confirms in general the above conclusions of Stotsenburg, though in a late paper he is inclined to believe that castration of male rats causes a slight relative increase in body weight over the control males; this, however, is not more than 3 per cent. to 5 per cent. And in spayed females there is both an increase in relative body weight and body length.

Livingston ('16) studied the effect of castration and spaying in the rabbit, but it is difficult to properly interpret his data, since the relative ages of his animals were unknown. Furthermore, he states that at the time of operation the ages varied from a few weeks to about one year, and that body weights ranged from 300 grams to more than two kilos, yet averages were taken on the group as a whole. In his Fig. 7 (curves 13 and 14), however, in which normal males controlled operated males and normal females the operated females from the same litter (ages not given), the indications are that the castrated males increase in weight, compared with the normal males, for a period of four months after operation. One is unable to judge, however, the comparative ages of all concerned and undoubtedly this would make a considerable difference in the results. This writer, as well as Hatai, believes that after castration there may or may not be a hypertrophy of the hypophysis, depending upon unknown factors; if hypertrophy does not take place, the animal undergoes a relative increase in weight, whereas there is an absence of a relative body weight increase if the hypophysis does not so react. If this is later proven to be correct, one will be unable to properly interpret weight differences following sex-gland removal without considering the possibilities of secondary influences dependent upon the hypophysis. One is led to believe from the experiments of Livingston with the rabbit that totally spayed females are hindered in growth; his curves show the normal female weight to be above that of the spayed females.

Thus Stotsenburg and Hatai are in practical agreement that the testis does not influence the growth of the rat, whereas in the rabbit Livingston believes that the testis retards growth; castrated animals are heavier than controls. My own experiments show but little effect in the guinea pig from castration, though there is a slight indication that the testis stimulates growth. As to the effects of the ovary on growth, Stotsenburg and Hatai agree that elimination of the rat ovary results in relative increases in the growth curves; Livingston, however, believes that ovariotomy lowers the growth curves in the rabbit. In the guinea pig my experiments show a slightly reduced growth curve for the spayed females up to the end of the first year.

IV. Body Length of Normal and Operated Animals.

The total body length of each animal was determined as each was killed on the 360th day, and it appeared desirable to record these observations for the bearing they may have on growth in general. The body length of each animal is given in Table II., as well as the average lengths for the different groups as a whole. From the average of each group one sees that the groups have essentially the same relationship to each other as the average weight determinations—*i.e.,* in the order, normal males > normal females > spayed females > castrated males. This indicates that the weight as a whole is due to a general growth rather than in certain groups to excess fat deposition as a secondary result from some specific action of the glands of internal secretion.

V. WEIGHT OF THE HYPOPHYSIS.

According to many investigators there is a more or less specific effect upon the hypophysis from gonadectomy; however, many of the conclusions found in the literature are diametrically opposed. It appears that too often merely gross weight comparisons of this small gland from two animals are used rather than a comparison of percentages of body weight; where small numbers of animals are used the latter method would offer more adequate means of studying specific reactions of the gland.

TABLE II.

SHOWING INDIVIDUAL WEIGHTS OF ORGANS OF INTERNAL SECRETION AND LENGTHS OF BONES AND THE AVERAGES FOR THE SAME FOR THE VARIOUS GROUPS.

Animal No.	Body Wt., Grams.	L'gth. Cm.	Hypophysis, Grams.	Thyroids, Grams.	Adrenal, Grams.	Spleen, Grams.	Femur, Cm.	Tibia, Cm.	Fibula, Cm.	Sex. Gl., Grams.
					Normal Males					
X1.....	980	31.6	.0116	.1646	.7300	1.0084	4.64	5.01	4.16	4.4406
X2.....	835	30.0	.0192	.2116	.8118	1.0225	4.62	5.08	4.30	3.9619
X3.....	870	30.4	.0152	.1642	1.1260	.6178	4.57	4.855	4.15	3.7612
X4.....	967	32.9	.0192	.1710	.7419	.6899	4.77	5.135	4.35	5.3212
X5.....	575	29.5	.0136	.1088	.5208	.7012	4.525	4.80	4.045	2.0410
X6.....	950	30.9	.0072	.1421	.9566	1.1141	4.51	4.95	4.100	5.9152
X7.....	810	27.5	.0144	.1402	.8900	.9570	4.46	4.87	4.16	4.6440
X8.....	1,120	32.0	.0150	.1886	1.3630	.9094	4.715	5.13	4.28	6.5590
X9.....	650	29.7	.0116	.1270	.6872	.5485	4.465	4.86	——	2.8073
X10....	550	27.0	.0104	.1330	.7433	2.9542	4.37	4.70	3.67	2.4573
X16....	885	30.6	.0147	.1306	.6580	.7095	4.645	5.00	4.24	4.2510
X22....	1,280	33.0	.0170	.1400	.7152	1.1344	5.095	5.61	4.70	5.0086
Average.	872	30.4	.0140	.1518	.8286	1.0305	4.615	5.00	4.196	4.2640

Castrated Males

X11....	555	28.0	.0018	.1546	.5200	.4034	4.4	4.765	——	
X13....	850	29.0	.0121	.1829	.7595	.8134	4.495	4.855	4.10	
X14....	940	28.5	.0154	.1826	.7100	1.6100	4.40	4.85	4.135	
X18....	830	29.0	.0130	.1208	.8598	.7920	4.45	4.95	4.205	
X20....	695	28.0	.0141	.1786	.7154	.8522	4.475	4.875	4.140	
X23....	995	29.5	.0170	.1700	.9584	.9516	4.780	5.185	4.380	
Average.	810	28.6	.0122	.1649	.7538	.9037	4.5	4.915	4.192	

Animal No.	Body Wt., Grams.	L'gth. Cm.	Hypophysis, Grams.	Thyroids, Grams.	Adrenal, Grams.	Spleen, Grams.	Femur, Cm.	Tibia, Cm.	Fibula, Cm.	Sex. Gl., Grams.

Normal Females

Y1.....	807	29.7	.0165	.2180	.7054	1.3174	4.37	4.70	3.93	.1302
Y2.....	880	30.1	.0035	.1610	.7793	.7526	4.47	4.92	4.195	.1918
Y3.....	925	29.2	.0142	.1372	1.0010	1.3911	4.375	4.725	4.015	.1766
Y4.....	875	29.1	.0164	.1710	.5230	1.3171	4.31	4.68	4.01	.1434
Y8.....	900	30.6	.0124	.1246	.6050	.7596	4.49	4.95	4.21	.1294
Y9.....	800	29.5	.0174	.1165	.7721	1.0550	4.47	4.945	4.265	.1784
Y10....	890	29.3	.0162	.1204	.5314	.9710	4.455	4.78	4.06	.1050
Y19....	975	29.0	.0170	.1873	.7169	.9610	4.57	4.95	4.22	.2158
Y20....	785	29.0	.0166	.1742	.5708	1.3666	4.575	4.865	4.125	.1552
Average.	871	29.5	.0150	.1567	.6894	1.0990	4.454	4.835	4.114	.1584

Spayed Females

Y12....	995	29.5	.0144	.2146	.6645	1.2505	4.61	5.14	4.345	
Y14....	915	29.0	.0134	.2096	.6407	1.1181	4.30	4.8	4.100	
Y15....	700	29.2	.0142	.1650	.5416	1.1551	4.595	5.15	4.300	
Y16....	850	29.2	.0164	.1718	.5418	.8070	4.68	5.06	4.350	
Y17....	800	27.0	.0128	.1412	.5286	.8470	4.50	4.91	4.220	
Y18....	800	28.5	.0119	.1534	.3540	.9126	4.55	4.95	4.240	
Y22....	925	29.0	.0118	.1498	.5760	.8744	4.56	5.05	4.300	
Y23....	1,045	29.5	.0124	.1348	.6456	.7556	4.70	5.07	4.345	
Average.	879	28.8	.0134	.1675	.5616	.9625	4.562	5.01	4.275	

In my own experiment the average gross weights of several glands from animals of the same age, sex, and operated condition form the basis of the comparison, the average body weights of the same lots of animals being known. Table II. gives the weight of individual hypophyses as they were removed from the animal on the 360th day of life. The weights of all the hypophyses for each group were averaged and a comparison of these average weights should give as good an indication of the reactions, if any, as any method of comparison that could be used.

Sexual Differences.—Comparing the average weight of the twelve normal male hypophyses (.0140 gram) with the average of nine normal female glands (.0150 gram), we see that the female hypophysis is 7.1 per cent. heavier than that of the male. And comparing the average body weights of the same two groups, one sees a difference of but one gram; in other words, the average body weights of the two groups are almost identical. There appears, therefore, to be a slight sexual difference in the weight of the male and female guinea pig hypophysis at the end of one year's growth, the female hypophysis being slightly heavier than the male.

Castration.—Comparing the average weights of the twelve normal male hypophyses (.0140 gram) with the average of the six castrated male hypophyses (.0122 gram), we find that the normal male hypophysis is 14 per cent. heavier than the hypophysis of castrated males. Comparing the body weights of these two groups, the normal males are 7.6 per cent. heavier than the castrated males, or, in other words, the relationship of the hypophysis of the normal male to that of the castrated male is proportionally greater than the relationship of the body weights. Thus we conclude that castration causes a relative decrease in the hypophysis as well as an actual one, as shown by the figures in the table.

Spaying.—If one compares the average weight of the hypophyses of the nine normal females (.0150 gram) with the average weight of the same gland of the eight totally spayed females (.0134 gram), it will be seen that the normal female hypophysis is 12 per cent. heavier than the hypophysis of the spayed females. And looking at the ratio of body weights of the two groups, it will be seen that the spayed females are approximately 1 per cent. heavier than the normal females. Thus at the end of one year the normal female hypophysis is both relatively and actually heavier than that of the spayed females, or, in other words, total spaying causes a decrease in weight of the hypophysis.

Discussion.—It has been impossible to find in the literature more than one or two observations wherein the weight relations between the hypophysis of the normal male and female of any group of animals have been determined. It would appear that a knowledge of sexual differences would be of great value in attempting to unravel the existing relations between the sex glands and the hypophysis. Hatai ('13) has shown that the hypophysis of the female albino rat is more than twice the weight of that of the male, and that in the Norway rat weight difference is also constant, but of less degree than in the albino variety. As noted above, the female guinea-pig hypophysis was heavier than the male gland, though the difference in this case is but 7 per cent. Too few determinations have been made, however, to warrant the conclusion that the same result, in general, would hold for other animals.

There is a great amount of controversial evidence on the effects of gonadectomy on the hypophysis, not only among the different groups of animals investigated, but also for the same group investigated by different workers. Fischera ('05) maintained that the hypophysis increased very materially in weight when gonadectomy was performed on the common fowl, cattle, rabbit, guinea pig, and buffalo. Marrassini and Luciani ('11), however, denied an increase in the hypophysis under these conditions for practically the same group of animals utilized by Fischera. And since the publications of the latter an increase in the hypophysis following gonadectomy has been reported for the female dog and rabbit (Parhorn and Goldstein, '05), male dog and rabbit (Cimorini, '08), male and female rat (Hatai, '13); but an increase of the hypophysis has been denied for the male guinea pig (Pirsche, '02), the male of the common fowl, cattle, dog, rabbit, guinea pig, sheep, and the female guinea pig (Marrassini and Luciani, '11). These very different results are undoubtedly due to inadequate controls. It is very difficult to judge, in most instances, if the conclusions drawn are warranted by the materials considered; even if they are, the majority of observations have been made upon an inadequate number of animals, many times even upon animals of a different stock. It appears that the observations of Hatai stand almost alone in respect to the materials used, for in this investigation the growth relations are sufficiently understood and conducted upon a sufficient number of animals to warrant an adequate comparison.

Without attempting to discuss the ideas of the various writers, advanced as explanations of their own particular results, I will but mention a correlation between the sex glands and the hypophysis first mentioned by Hatai and approved by Livingston. Hatai called attention to an apparent reciprocal relationship between these two glands, of such a nature that different effects can be noted in subsequent growth. He noted that castrated animals showing an hypophyseal hypertrophy did not show an overgrowth in body weight, and conversely if the hypophysis had not increased in weight, relative to the normal, the animal did show an overgrowth in body weight. "Thus if a compensatory growth of the hypophysis does not follow, as is the case after spaying, the product of the unaltered gland must be employed for two purposes: one, to replace the ovarian hormone, and, two, for the normal uses, whatever they may be."

Later experiments led Hatai to believe that his assumptions were correct, as further observations substantiated the idea. Livingston ('16) agrees with the assumption of Hatai inasmuch as the results of his series of gonadectomized rabbits could be interpreted on this basis. He states that when the body responds by an increase in weight, even though slight, the pituitary does not show a compensatory hypertrophy.

My own observations on the guinea pig, however, do not accord with the idea that in the absence of hypophyseal hypertrophy an overgrowth of body proportions follow. The average weight of the normal male hypophysis was 14 per cent. greater than that of the castrated male, whereas the average body weight of the castrated males was less than that of the normal animals; and similarly the average weight of the normal female hypophysis was 12 per cent. greater than that of the spayed females; and, again, the average total body weight of the spayed female was below, or almost identical with, that of the normal female. And in each of the above cases, to substantiate the hypothesis, there should have been a relative increase in the body weight of the gonadectomized animals, since the hypophyseal weight was less. It remains, therefore, for subsequent investigation to prove that there is this general relationship that has been indicated by the rat and rabbit material.

VI. Growth of Thyroids.

The actual weights of the thyroid glands of each animal of the series are shown in Table II. and the average weight of the glands of each group as well (both thyroids weighed in each case).

Sexual Differences.—When the average weights of the twelve normal male thyroids (.1518 gram) are compared with the average weights of the nine normal female glands (.1567 gram), we see that the normal female thyroids are approximately 3.2 per cent. heavier than those of the normal males. Since the total body weights of the two groups do not vary more than one gram, it appears that the female guinea-pig thyroids are heavier than the male glands.

Castration.—Comparing the average thyroid weights of the normal males (.1518 gram) with those of the castrated males (.1649 grams), the thyroids of the castrated males appear to be 8.6 per cent. heavier than those of the normal animals. But since the body weight of the normal males is 7.6 per cent. greater than that of the castrated males, the relative differences of thyroid weights are really considerably greater than the differences of their actual weights. Hence we may conclude that castration in the guinea pig favors the growth of the thyroids.

Spaying.—The average weight of the normal female thyroid (.1567 gram) compared with the average of the spayed females (.1675 gram) reveals a difference of 6.8 per cent. in favor of the spayed animals. Since the averages of body weight favor the spayed females by only 1 per cent., we see that elimination of the ovaries favors growth of the thyroids in the females.

To the writer's knowledge there is no authenticated observation of a sexual difference in the weight of the thyroid gland in any animal. It must be emphasized that results obtained from a comparison of one or two animals are decidedly unreliable and of little value.

In all cases where a sufficient number of animals of corresponding ages have been compared the variability in weight is such that results of a definite character are not indicated. Thus Livingston ('16) determined the weights of the thyroid glands in a large number of rabbits, normal and operated, but the degree of weight variability was so pronounced that no definite conclusions were warranted. Hatai ('18) definitely states that there is no apparent sexual difference in the weight of the thyroids in the rat. Furthermore, the variability in thyroid weight was so pronounced among the operated rats that results of a definite character were not indicated.

In the guinea pig comparison of the average weights of male and female thyroids at the end of one year favored the females to a slight extent.

Gonadectomy apparently favors the growth of the thyroid in the guinea pig inasmuch as each operated group showed a definite increase in the thyroids at the end of one year. However, in view of the many failures to establish a definite effect, one must be very skeptical indeed in applying these results as a general effect of gonadectomy. Obviously repetition of the experiment is necessary before general principles can be held as established even for the guinea pig.

VII. Growth of Adrenals.

The individual weights of the adrenals (two in each case) can be seen by reference to the different groups given in Table II.

Sexual Differences.—When one compares the average weight of the normal male adrenals (.8286 gram) with the average weight of the normal female adrenals (.6894 gram), it is apparent that there is a considerable difference in the two sexes; the normal male adrenal weights are approximately 20 per cent. greater than those of the normal females, the average of the body weights of the two sexes being almost identical.

Castration.—The average normal male adrenals (.8286 gram) compared with the average castrated male adrenal (.7538 gram) is 9.9 per cent. heavier. However, when average body weights are compared, one sees that the normal male is 7.6 per cent. heavier; the relative difference, therefore, is seen to be very small, though favoring slightly the normal animals. It appears, therefore, that castration inhibits the growth of the adrenals to a slight extent.

Spaying.—When the average weights of the normal female adrenals (.6894 gram) are compared with the average weights of the spayed female adrenal (.5616 gram), one sees that the normal female adrenal is approximately 22.7 per cent. heavier than those of the spayed female. Since the body-weight comparison shows a difference of but 1 per cent., the conclusion is that spaying is followed by an inhibition of the growth of the adrenal gland.

Aside from the present data for the guinea pig there is apparently but one other set of data that gives an adequate comparison of the suprarenal weights in the two different sexes. Livingston's data ('16), consisting as it does of suprarenal weights at different ages and conditions of life, is inadequate for an intelligent comparison. Hatai ('15), however, found for the rat a marked sexual difference in the weights of the suprarenal glands. The female glands are approximately double those of the males in the mature animals. For the guinea pig there is also a decided sexual difference in the suprarenal weights, but in these animals it is the male that is the larger. At the end of one year the male suprarenals are approximately 20 per cent. greater in weight than those of the female.

Certainly to understand the general relationship existent between the suprarenals and the sex glands more data are desirable; particularly is this true for the guinea pig, in which the number of animals is inferior to that of the rat. Obviously it is useless to attempt an analysis of the conditions until the facts are well established.

One may well conclude that the same is true for the effects derived after gonadectomy, and particularly so in view of the many discrepancies noted in the literature. The fundamental conditions of the various experiments are so varied that only divergent results can be anticipated. Soli ('09) reports a decrease in the relative weight of the suprarenals after castration in guinea pigs, rabbits, and chicks, but the number of animals observed was small. Marrassini and Luciani ('11) reported considerable data on the suprarenal weights in both sexes of the rabbit and guinea pig after gonadectomy, but in their experiments the time limit was too brief for complete changes to have been registered. Castrated and spayed males and females were compared with normal animals of approximately the same age, but the operated animals were killed in all cases within ninety days after the operation, and several were allowed to live but fifteen days after gonadectomy. The majority of the cases compared indicate a tendency toward a suprarenal hypertrophy in both sexes of the rabbit and guinea pig. Hatai ('15) found that the suprarenal of the male rat increased materially in weight after castration, whereas the female suprarenal showed a decrease of from 5 per cent. to 25 per cent. compared with normal females. In the guinea pig the relative weight of castrated and normal male suprarenals in my experiments are but little different. It appears, however, that there is a slight reduction in the weight of these glands in the castrated animals. In the spayed females the suprarenals were approximately 22 per cent. lighter in weight than those of the normal females.

The fundamental relationship between the sex glands and the suprarenals may be actually different for different types of animal forms, as is indicated by the discordant results reported. But it is undeniable that more exact data are necessary before an approach to the truth can be formulated. For the experimental data to be of value comparisons must be made with animals of similar ages and existing under similar living conditions. It is obvious, also, that data based upon considerable numbers of operated cases are of much greater weight than that obtained from a random comparison of a few animals.

VIII. Growth of the Spleen.

While the spleen is not ordinarily considered an organ of internal secretion, it appeared desirable to include the weights of this organ in the various animals, as all such data may prove of value at a later time.

Sexual Differences.—Comparing the average weight of the normal male spleen (1.0305 grams) with that of the average of the normal female spleen (1.0990 grams), one sees that the normal female spleen is 6.6 per cent. heavier than that of the male; since the average body weights are the same, we may conclude that there is a slight sexual difference in the spleen favoring that of the female.

Castration.—Comparing the average weight of the normal male spleen (1.0305 grams) with the average weight of the castrated male spleen (0.9037 gram), a slight difference is evident. The normal male spleen is 14 per cent. heavier than the castrated male spleen, but the body weight of the normal male is 7.6 per cent. greater than that of the castrated male; the relative difference is therefore not so great. Apparently castration slightly inhibits growth of the spleen.

Spaying.—Comparing the normal female spleen with that of the spayed female spleen, we note that the spayed female possesses on the average a spleen 14 per cent. less in weight than the normal; since the body weights are but slightly different, we may conclude that spaying results in a decrease in the spleen growth.

IX. Bone Lengths.

Reported increases in the length of bones following gonadectomy indicate that the sex glands control to some degree the lengths acquired. In order to determine possible differences referable to a sex-gland disturbance, the bones of the hind leg of each animal of the series were measured at the time they were killed. These measurements are given in Table II. and are expressed in average lengths of the two similar bones from each animal. Thus the given femur length for each animal is the average between the lengths of the two femurs, etc., and it has been found that differences between the two bones are often greater than could be anticipated.

Sexual Differences.—When a comparison was made between average lengths of the hind-leg bones of the normal male group and an average of similar bones from the normal female group, it was found that the bones of the normal males were but slightly greater in length than those of the females. The difference was as follows: femur, 3.6 per cent. longer; tibia, 3.4 per cent. longer; fibula, 1.9 per cent. longer.

The sexual difference in bone lengths of normal guinea pigs is therefore one of slight degree, the males being favored as to growth in length.

Castration.—When the average lengths of the bones of the normal males were compared with similar ones of the castrated animals, the actual lengths were greater in the normal animals than in castrated ones; the femur was 2.5 per cent. greater in length, the tibia 1.7 per cent., and the fibula considerably less than 1 per cent. greater in length than the corresponding bone of the castrated male. This actual difference, however, is minimized when it is remembered that the total body length of the normal males is approximately 6 per cent. greater than that of the castrated animal. If we compare the length of the bones as percentages of total body length, we find that each bone of the castrated animal is *relatively* longer than the corresponding bone of the normal animal (length of femur, as percentage of total body length, norm. 15.18 per cent., castrated 15.73 per cent.; tibia, norm. 16.44 per cent., cast. 17.18 per cent.; fibula, norm. 13.8 per cent., cast. 14.3 per cent. Since the relative lengths of the bones are greater in the castrated animals, the conclusion is that castration favors slightly growth in length of hind-leg bones. It must be emphasized, however, that this increase is not marked and careful computation must be employed to make it apparent. It appears so insignificant that little value is attached to it, and certainly the results are not in agreement with those who maintain that castration results in a marked overgrowth in bone lengths.

Spaying.—When the average bone length of the normal female group were compared with those of the spayed female group a decided difference in length was noted; the bone length of the spayed females was greater than similar bone lengths of the normal females. The observed length differences were as follows: femur, 2.4 per cent.; tibia, 3.6 per cent., and fibula 3.9 per cent. longer in spayed females than in normal females. When one considers that the total body length of the normal female was 2.4 per cent. greater than the spayed female, it is apparent that the *relative length* difference is greater than that of the *observed length.* We may conclude from this data that spaying in the guinea pig is conducive to greater lengths in hind-leg bones.

A number of investigators have reported exaggerated bone growth after gonadectomy, particularly in the absence of the testis. Poncet ('03), presenting a résumé of his own earlier experiments, as well as those of Pirsche ('02) and others, maintains that castration is followed by a general increase in the size of the skeleton. Observations on eunuchs, clinical cases of testicular atrophy, and experiments on laboratory animals were cited. In reference to the guinea-pig bones, he found an increase in length of 3 mm. for the femur and 4 mm. for the tibia in castrated animals; the total lengths were not given, consequently the relative growths of bone in the two cases are unknown. In capons he found an increase of 8 mm. for the femur and 1 cm. for the tibia. Delay in the ossification of the epiphyses following castration is considered the causal factor for increase in length. Sellheim ('99) found delayed ossification in castrated vertebrates (pig, dog, and bull) and a consequent increase in size and length of bones. Hatai ('15) found a very slight increase in the ratio of body length to bone length in castrated rats. He mentions, however, that this difference appears only upon close computation, and is in doubt whether any significance should be attached to his findings.

X. Discussion.

The primary object in conducting the experiment reported herein was to study the reactions in weight of guinea pigs, from birth to maturity, as this may be influenced by the sex glands. Certainly secretions of the sex glands do modify somatic structures in many vertebrates and it has been assumed that weight of laboratory animals such as the rat and the guinea pig reflect, to some degree, their sexual nature; the differences are supposedly detectable if comparisons are made between the weights of normal animals and those having undergone operations at a previous date. However much one may be inclined to doubt the advocacy of utilizing weight as a criterion of sex-gland conditions, an intimate understanding of the reactions are necessary before the doubt can be expressed in definite form.

In the guinea pig (speaking of averages of groups of animals of the same age, same conditions as concerns sex glands, and reared under identical conditions) normal males are constantly heavier than normal females up to the end of the first year; at this time the weights between the two groups are almost identical and, according to Minot, the females subsequently become slightly heavier than the males. But does this mean that the testis promotes, and the ovary retards, growth and increase in weight? Elimination of the gonads of each sex should afford a basis for a partial answer to the question by comparing the growth curves of each group with that of the normal group. Referring to Fig. 1, it is apparent that the gonadectomized groups fall below the curves of the normal groups for the greater part of the year; but by the 300th day the spayed females have attained the weight of the normal female and remain slightly above these until the close of the experiment. At the end of the experiment, therefore, the spayed females are heavier than normal females, whereas the castrated males are lighter in weight than the normal males. This appears to indicate that the testis does favor growth (absence reduces weight), and that the ovary retards growth (normal females lighter). But let us apply this evidence using as the basis of our comparison four females of the same litter, Y17, Y18, Y19, and Y20. These four sisters, reared together in the same cage, each show gradual growth throughout the year with an entire absence of temporary losses in weight; two had been spayed (Y17, Y18), while the other two remained normal females. On the 360th day each of the two spayed animals weighed 800 grams, one normal female (Y19) weighed 975 grams, and the other (Y20) weighed 745 grams. Since removal of the ovaries causes a relative increase in weight (when averages of entire groups were compared), the spayed females should weigh more than the normal ones. But referring to Table I., it becomes apparent that Y19 (normal) is 22 per cent. heavier than the spayed sisters, whereas Y20 is 2 per cent. lighter than the operated sisters. Our supposition, therefore, is proven that elimination of the ovary leads to relative increase in weight if the comparisons are made between these operated animals and Y20, but it is disproven if the comparisons are made with Y19 representing the normal condition. Consequently, if individual variations in weight are so great with animals of the same litter and lead to such discrepancies in results, one could not expect a more adequate basis of comparison if the animals of unknown history are chosen from the stock at random. One can not fail to be convinced of the magnitude of variation in weight in animals of the same age, sex, and under identical conditions if the weights on the 360th day are compared (Table I.); in some cases the individual variation is greater than 100 per cent. If, therefore, this group of total weights demonstrates nothing more (and indeed little is claimed for it), certainly it shows the fallacy of comparing at random the weights of two, three, or four animals and claiming for it partial proof for an hypothesis. It appears that the claim of the writer is not only justified, but proven, that the weight of an animal as a character indicating the effect of a sex-gland graft is not only worthless as scientific data, but very confusing to the entire problem of the modifying effects of gonads.

In reference to the effects of gonadectomy on the weight of the glands of internal secretion, little will be added to the discussion in connection with each section of the paper. The great discrepancies in results, even diametrically opposed conclusions, of different investigators working with the same groups of animals have already been pointed out. The chief difficulties in adjusting the results from the various sources appear to center around inadequate controls for the experiments. Should one desire to study the effects of gonadectomy on such a structure as the hypophysis, it should be obvious that animals of similar strains, reared under similar conditions, operated at similar ages, and controlled by the proper groups are essential. Variations in general metabolism are not only so great among animals of different ages and strains that isolated comparisons often indicate inconsistent results, but even animals of the same litter may show marked differences.

The writer does not wish to imply that this experiment has been conducted in an ideal manner, but an attempt has been made to know, in so far as is possible, the history of the animal before and after operation, and to afford comparisons of groups of considerable size rather than mere isolated comparisons. Furthermore, it is realized that unknown conditions may have influenced the results herein reported and repetition is highly desirable before they can be accepted as of particular value.

As to the influence of gonadectomy on bone growth, the above criticisms apply as well. Growth in general body proportions is to a certain extent relatively constant for various parts. It should be supposed at once that a somewhat larger animal than a brother or sister would be expected, in general, to have longer bones and yet not show a specific reaction in bone growth conditioned by the presence or absence of sex-gland secretions.

The results of an analysis of the bone lengths of this group of animals is surprising from the lack of more appreciable differences. In fact, the most clearly disproportionate length occurred in the spayed females. The bones of this group seem to reflect a specific effect of the absence of the ovarian secretion. The literature leads one to believe that differences between normal male and castrated male bone lengths is pronounced and easily detected. But the difference in lengths among the different groups of this experiment was so slight that careful computation was necessary to reveal it; the difference was so small that it appears almost insignificant.

Striking as may be the influence of the internal secretions of the sex glands on some characters in certain animal forms, it appears difficult and often impossible to discover characters in ordinary laboratory animals that are of sufficient difference and constancy in the two sexes to be capable of analysis by experimental procedure. And many of the characters cited in the literature supposedly offering a demonstration of the power of sexual secretions to effect modifications in the opposite sex fall to the ground if subjected to critical analysis. In the writer's opinion the character of weight reactions in guinea pigs belong to this group.

XI. Summary and Conclusions.

On the basis of observations on the guinea pig, herein reported, the following conclusions are drawn:

1. The curve of growth for normal male guinea pigs is consistently above that for normal females up to the end of the first year, when the two curves practically coincide.

2. Gonadectomy is followed by a decrease in the growth curves for both sexes; however, by the 300th day spayed females have reached the weight of normal females, and at the end of one year are 1 per cent. heavier than the normals.

3. The relative weight of a guinea pig is worthless as an indication of its sexual condition; properly controlled groups of weights may offer a debatable criterion of sex-gland effects, but random comparisons indicate nothing.

4. Total length of the animals correspond to total weights in the order: normal males > normal females > spayed females > castrated males.

5. The relative weight of the hypophysis of normal females is slightly greater than that of the normal males at the end of the first year.

6. Gonadectomy is followed by a relative reduction in weight of the hypophysis in operated males and females.

7. Thyroids of normal females are slightly heavier than those of normal males.

8. Gonadectomy appears to favor growth of the thyroids in both operated males and females.

9. Adrenals of normal male guinea pigs are approximately 20 per cent. greater in weight than those of normal females.

10. Gonadectomy is followed by a relative decrease in weight of adrenals in both sexes; though slight differences appear in males, the effect is considerably more pronounced in females.

11. The normal female spleen was found to be slightly heavier than that of the normal male.

12. Gonadectomy appears to cause a reduced growth of the spleen in both sexes.

13. The length of the hind-leg bones of the normal male is slightly greater than that of the normal female.

14. Castration favors, to a slight extent, the growth of leg bones in operated males, though the differences appear to be relatively insignificant.

15. Spaying is conducive to bone growth in operated females; the relative differences are considerably more pronounced than those following castration.

16. Repetition of the experiment is deemed highly advisable before the results, herein reported, are accepted as indicative of general principles.

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BIOLOGICAL BULLETIN

STUDIES ON THE CORRELATION BETWEEN METABOLIC GRADIENTS, ELECTRICAL GRADIENTS, AND GALVANOTAXIS. I.

L. H. HYMAN AND A. W. BELLAMY,

HULL ZOÖLOGICAL LABORATORY, UNIVERSITY OF CHICAGO.

I. INTRODUCTION.

Several years ago, while investigating the physiology of regeneration in oligochætes with the aid of the susceptibility method, the idea occurred to the senior author that there was a remarkable similarity between the susceptibility differences and bioelectric differences. The points of similarity noticed were the following:

1. Any injured region or surface is more susceptible to toxic agents than uninjured regions. It has long been known that injured regions are electronegative (galvanometrically[1]) to uninjured ones.

2. Increase in activity increases the susceptibility and, as is well known, induces an electronegativity (galv.).

3. There is a susceptibility gradient along the axes of organisms, such that in general the anterior end is the most susceptible and the susceptibility decreases along the principal axis. It was known at that time for a few animals that there is also an electrical gradient along the axis, the anterior end being the most negative

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[1] It has unfortunately become customary to refer to the bioelectric currents with regard to the direction in which they run in the galvanometer, which is of course the reverse of the direction in the organism. In order to avoid any possibility of confusion with respect to the direction of the current, the abbreviation "galv." in parenthesis will be employed to indicate the direction of the current through the galvanometer, and the abbreviation "int." to indicate the direction of the current through the animal. In all of the tables the designations negative and positive refer to the direction of current with respect to the galvanometer.

(galv.) and the negativity diminishing along the antero-posterior axis.

4. In the oligochætes the susceptibility gradient was found to be of a characteristic kind (Hyman, '16; Hyman and Galigher, '21). Both anterior and posterior ends are the most susceptible and the susceptibility decreases from both ends toward the middle. It is a very suggestive fact that the electrical gradient in oligochætes is in agreement with this susceptibility gradient. As first shown by Morgan and Dimon ('04), the anterior and posterior ends of the earthworm are electronegative (galv.) to the middle. This remarkable agreement between the electrical and susceptibility gradients indicates strongly that they have a common physiological basis.

From a consideration of the facts mentioned above the senior author was led to believe that the bioelectric currents are due to the same factors which are responsible for the susceptibility differences. The susceptibility differences are known to be correlated with metabolic differences. Organisms or parts of organisms which are highly susceptible to toxic agents have a higher metabolic rate than those which are less susceptible. Susceptibility is increased by factors which are known to increase metabolic rate and decreased by those which lower metabolic rate.

It thus appears that the bioelectric currents are correlated with differences in the rate of chemical activity at different levels or in different parts of an organism or tissue, probably chiefly with differences in the rate of oxidation. In general, any region of increased chemical activity becomes *ipso facto* electronegative (galv.) to any region of lower chemical activity. Injury increases the rate of chemical change (cf. Tashiro, '17) and the injured area develops an electronegativity (galv.). Activity of muscle, nerve, gland, etc., is generally accompanied by an increased rate of respiratory exchange; hence is also characterized by the appearance of an electronegativity. When the differences in rate of chemical change are temporary, the accompanying bioelectric currents are also temporary, as in the case of the current of action; when the differences are permanent, the bioelectric current is likewise permanent, as in the case of the electrical gradient which exists along the axes of organisms.

While it appears probable that differences in the rate of oxidation are generally responsible for the origin of potential differences in organisms, this need not necessarily be the case. Other chemical reactions could also give rise to potential differences, and in such cases the direction of the resulting current might be different. That is to say, where an oxidative reaction is the cause of the current, the region of higher rate becomes electropositive (int.), since oxidation consists in the assumption of positive charges (or the loss of negative charges). On the other hand, if some other chemical reaction were concerned, the direction in which the current would pass naturally would depend on the nature of the reaction, and could not be predicted until the reaction involved were known. It is very likely that in some organismic activities a bioelectric current would be produced in which the region of highest rate of activity or region of stimulation would be electropositive (galv.) to less active or unstimulated regions. It is also, of course, possible that differences, not only in the rate, but also in the kind of chemical reaction at different regions, may give rise to potential differences, in which case again the direction of the current will depend upon the reactions involved.

Our general point of view, then, is that potential differences in living things usually originate in metabolic processes, probably chiefly the oxidative processes, since oxidation-reduction reactions give rise to currents identical as to direction with those occurring in organisms. Such currents could arise either through the presence of an oxidation process at one point accompanied by a reduction process elsewhere or through a difference in the rate of the oxidation-reduction process at different regions, giving rise to a concentration chain. Although we are unable in the present state of our knowledge to decide between these two possibilities, it seems more probable that the potential differences are due to the second of the two suggestions, since in the organism the oxidation process is not, as far as we know, separated from the reduction process. Differences in the concentrations of ions at different regions would therefore seem to be the usual sources of the potential differences; such concentration differences arise through differences in the rate of reaction.

The point of view presented here that the bioelectric currents are chemical in origin has been held by a number of physiologists.

It seems to have originated with Hermann, who, in 1867, formulated it with a clarity and simplicity which scarcely permit of any addition. As the publication in question is not readily obtainable, it seems worth while to quote Hermann's views *verbatim*. Hermann based his conceptions on his discovery ('67*a*) that the chemical processes in muscle contraction and muscle rigor are identical. He postulates that these chemical processes consist chiefly of a splitting, designated by him as Spaltung, of chemical substances previously present in the muscle. On this basis he proceeds to develop his views concerning the origin of the muscle and nerve currents as follows ('67*b*):

"Wir nehmen also an, dass die Muskelsubstanz in der Ruhe in beständiger langsamer Spaltung begriffen ist, und dass die Muskelreize die Spaltung momentan sehr bedeutend beschleunigen (p. 4). . . . Durch das Anlegen eines Querschnitts beschleunigen wir also den im unverletzten Muskel äusserst langsam verlaufenden Spaltungsprocess local in hohem Grade (p. 5). . . . Aus Analogie und theoretischen Gründen ist es sehr wahrscheinlich dass die in schnellerer Spaltung begriffenen Muskeltheile sich negativ verhalten gegen solche welche in langsamerer Spaltung begriffen sind (p. 6). . . . Jede beliebige Stelle eines Muskels wird durch Erwärmung negativ gegen die übrige Muskelsubstanz (p. 9). . . . Von zwei beliebigen Muskelschichten verhält sich die in schnellerer Spaltung begriffene negativ gegen die andere, und zwar so stärker je grösser der Unterschied ihre Spaltungsgeschwindigkeiten ist (p. 22). . . . Der Muskelstrom muss ferner zunehmen durch Einflüsse welche dem vom Querschnitt her vorschreitenden Spaltungsprocess begünstigen, namentlich durch erhöhte Temperatur (p. 23). . . . Während der Contraction, die wir uns zunächst, der Einfachheit halber, gleichzeitig alle Theile des Muskels ergreifend denken, ist nach dem in Eingange Gesagten im ganzen Muskel eine Beschleunigung des Spaltungsprocesses vorhanden. In den bereits erstarrten Theilen des Muskels (*i.e.*, the cut surface) fehlt diese Beschleunigung. In den übrigen ist sie um so geringer, je grösser ihre Spaltungsgeschwindigkeit bereits in der Ruhe ist (p. 31). . . . Der polarisirende Strom beschleunigt jenseits der Electroden die Spaltung in der catelectrotonisirten und verlangsamt sie in der anelectrotonisirten Strecke. Diese Einflüsse nehmen mit zunehmender Entfernung von den Electroden ab (p. 38). . . . Der polarisirende Strom wirkt also an jeder Nervenstelle auf die Geschwindigkeit des Spaltungsprocesses und auf die Erregbarkeit in gleichem Sinne (p. 45). . . . Das erregter Zustand nichts ist als schnellerer chemischen Process, also Erregung nichts als der Act der Beschleunigung (p. 45). . . . Die Erregbarkeit einer Nervenstelle hängt ab von der Geschwindigkeit des Spaltungsprocesses in derselben. Die Erregung einer Nervenstelle beruht auf einer plötzlichen Beschleunigung des Spaltungsprocesses in derselben. Die Leitung der Erregung im Nerven beruht auf der Fortpflanzung einer plötzlichen Spaltungsbeschleunigung längs des Nerven (p. 46). . . . Der Muskel enthält eine Substanz welche langsam spontan sich spaltet in (unter anderen) Kohlensäure, ein fixe Säure und Myosin; letzteres tritt in gallertiger Lösung auf und wird erst unter Verkurzung fest nachdem es zu einer grossen Concentration gelangt ist. Der Nerv enthält ebenfalls eine under Säurebildung sich spaltende Substanz, welche aber kein dem Myosin entsprechendes Zerfallproduct liefert. Der Spaltung kann durch gewisse Umstände (Wärme, in Nerven Catelectrotonus) beschleunigt, durch ander (Kälte, im Nerven Anelectrotonus) verlangsamt werden. Je grösser die Spaltungsgeschwindigkeit voreits ist, um so geringer ist der Einfluss beschleunigender Einwirkungen auf die Geschwindigkeit. Jedes in schnellerer Spaltung begriffene Muskel- oder Nerventheilchen wirkt beschleunigend auf den langsameren Spaltungsprocess im Nachbartheilchen, um so energischer, je grösser der Unterschied in beider Geschwindigkeit ist. Jedes in schnellerer Spaltung begriffene Teilchen verhält sich negativ electrisch gegen ein in langsamerer Spaltung begriffenes. Diese Ströme bilden die Umkehrung der Wirkung des Stromes auf die Spaltungsgeschwindigkeit (p. 63).

From these excerpts it is evident that Hermann at that time regarded stimulation as an acceleration of metabolic rate and considered that any such acceleration is accompanied by the development of an electronegativity (galv.). This view is identical with that advanced in this paper and discussed in more detail in a previous publication (Hyman, '18). Subsequently, however, Hermann abandoned these conceptions, regarding them as "incomplete," and developed his alteration theory. The alteration theory, however, is not really a theory of the cause of the bioelectric currents, but merely a statement of the general laws of these currents.[2] The principal terms of the alteration theory are: that the uninjured resting muscle or nerve is isoelectric; that all potential differences arising in muscle or nerve are due to physiological alterations of their substance; that any injured place or any stimulated place becomes electronegative (galv.) to intact or resting regions; and that the electronegativity is the consequence of the injury or stimulation (cf. Hermann, '79, p. 235). These statements are now, of course, accepted facts of electrophysiology. An explanation of the source of the potential differences due to injury and stimulation was not included in the alteration theory, and Hermann (l.c., p. 240) was at this later period disinclined to consider the possibility of a chemical origin of the bioelectric currents.

A viewpoint very similar to the early conceptions of Hermann quoted above was, however, soon developed by Hering. This viewpoint is suggested in a publication by Hering in 1879, in which (p. 242) he proposes the possibility that "an der Stellen wo der Strom die contractile Substanz der Einselfaser betritt oder verlässt, eine chemische Alteration dieser Substanz stattfinden kann, welche in übrigen durchflossenen Strecke nicht statthat." Hering presented his conception of the metabolic origin of the bioelectric currents in 1888. In this paper Hering expresses the belief that all of the functions of living matter are metabolic—*i.e.,* chemical—in nature, either, in general, assimilatory or dissimilatory, and that alterations of chemical function are responsible for the bioelectric currents. Some typical quotations may be given (pp. 250–251): ". . . nearly twenty years ago . . . the prevailing conception of the facts of general nerve physiology was strictly physical. I had, therefore, to insist . . . that these functions were essentially chemical, and that the intrinsic chemical nature of the vital processes must not be overlooked in favor of their physical symptoms. . . . Hermann's weighty dictum of the isoelectricity of uninjured 'resting' nerve or muscle signifies to me that such a tissue develops no current that can be led off externally, *so long as its metabolism—i.e., internal chemical function—is equal in all parts. Every disturbance of this equilibrium sets up currents that can be led off.* Alteration of chemical function in any part of a living continuum may, however, be expressed, not merely in its becoming *negative,* but equally in its becoming *positive* to unaltered parts. Hence, if we are to characterize the point differing in chemical function from the rest of the substance, as (relatively) altered, we must, in my opinion, *distinguish between a (relatively) positive and a (relatively) negative alteration.* And it is not altered chemical composition, but altered chemical function, which may lead to altered composition, that characterizes this change. . . . If all parts of a living continuum are in equilibrium or alter at the same rapidity, ascending or descending,[3] there will be no current to lead off. Every difference in rate or direction of alteration, however, produces a current that can be led off. *We may, accordingly, conceive of all the different rates of positive and negative alteration as forming a series of such a character that the most rapid ascending alteration forms the upper—i.e., positive—the most rapid descending alteration the lower—i.e., negative—end of the series.* If two parts of a living continuum, which differ in chemical function, are joined by an external circuit, there will, *cæteris paribus,* be a stronger current in proportion as the distance between the two points connected in circuit is greater in the above series, and the positive current will always flow through the external circuit from the point nearest the positive end of the series to that nearest the negative end. *This is the universal law of all physiological currents in nerve and muscle."*[4]

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[2] The alteration theory may be considered as a theory as concerns the current of injury which it regards as due to alterations at the cut surface rather than to the exposure of a previously existing negativity at the cut surface. This latter point of view is presented by Bernstein ('12) and the question may be regarded as still open. However, alterations undoubtedly occur at the cut surface, due to the cutting, and it is rather more than likely that such alterations play a rôle in the current of injury, even though Hermann's crucial experiment, supposed to show that a measurable interval elapses between the cutting and the full development of the electronegativity, be discarded because of inadequate technic.

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[3] By the term "ascending" Hering means assimilatory metabolic change, and by "descending," dissimilatory metabolic change.

[4] The quotation is taken from the English translation in *Brain* with a few changes which follow the translation in Biedermann's "Electro-physiology." Italics of the original are preserved.

Biedermann ('96), a pupil of Hering's, adopts the latter's viewpoint, designating it as the Hermann-Hering theory. Biedermann likewise refers "all electromotive activities of living matter to chemical changes of the substance." Further details will be found in the volume cited, p. 351 ff.

Waller has long been an exponent of the theory of the metabolic origin of the bioelectric currents, which he designates as "blaze" currents because they are conceived of as due to sudden accelerations of normal chemical processes. This viewpoint appears clearly in his Lectures on Animal Electricity ('97), in which publication the following statements appear (Waller uses the terms positive and negative with reference to the tissue, not to the galvanometer—*i.e.*, the reverse of the usual usage): "Active matter is electropositive (int.) to inactive matter; more active matter is electropositive to less active matter; matter that is by any means stirred up to greater activity is rendered electropositive to undisturbed matter; matter whose action is lowered is electronegative to matter whose action is normal." Waller further goes on to say that any more active spot "where more chemical action is going on" is electropositive (int.) to a less active spot "where less chemical action is going on." A similar statement is made in his Signs of Life ('03), pp. 84–85: "A lump of protoplasm at rest and homogenous throughout is isoelectric throughout; let it be acted upon by its environment, any point of its surface is chemically more active" and becomes electropositive to "any point of its mass." If the mass becomes chemically more active than the surface, then the mass becomes electropositive to the surface.

In 1903 Mathews discovered the electric gradient along the axis of certain hydroids. This was the first case in which a correlation between potential differences and the principal axis was established for animals. Mathews suggested that the potential differences are due to metabolic differences, and that such potential differences may control polarity. Mathews's present views concerning the relation between the bioelectric currents and metabolic processes, according to personal conversation with him, coincide with those presented in this paper. Mathews's student Tashiro ('17) also favors the conception that "chemical changes underlie and produce the electrical changes" (l.c., p. 102).

Recently R. S. Lillie ('17, '19, '22, and others) in a number of interesting and suggestive papers has been inclined to attribute the origin of potential differences in organisms, particularly of the current of action, to local chemical change, which concerns chiefly the limiting membrane. This conception is added to Lillie's former views of the rôle of depolarization of the surface membrane in the causation of the current of action. Thus Lillie says ('22, p. 17): "Apparently any rapid local decrease of surface polarization (sufficient in range) causes stimulation in the typical irritable system such as muscle and nerve. This purely physical change, however, is merely the precursor or determinant of the local stimulation reaction; it is not the reaction itself. The latter is a physiological process dependent, like all such processes, on chemical reactions." Lillie is also further of the opinion that bioelectric circuits may be oxidation-reduction circuits.

The conception set forth in this paper, that potential differences in organisms originate in differences in metabolic rate, has been mentioned briefly in several publications from this laboratory—*e.g.*, Child, '15, pp. 63–64, and '21*a*, pp. 44–46. The senior author has also published a preliminary general paper (Hyman, '18) in which her views are more fully set forth than in the present paper and to which the reader is referred for further details.

The preceding pages attempt to give some account of the development of one idea concerning the origin of the bioelectric currents. The consideration of other suggestions concerning these currents involves the working over of a large literature and is deferred for the present. It may, however, be stated that there are two other principal suggestions as to the causation of these currents. One of them may be referred to as the concentration-cell theory and the other as the membrane-depolarization theory. These two ideas are more or less intermingled and most investigators who have supported the concentration-cell theory have postulated membranes to bring about the concentration differences. The idea that the bioelectric currents arise from concentration chains has been ably discussed by Bernstein, '02, '12; also by Cremer ('06), where earlier references will be found. Bernstein's analysis shows that there are three types of chemical chains setting free electricity: those in which the electric energy decreases with rise of temperature, those in which it does not alter with change of temperature, and those in which it increases with rise of temperature, so that the chain cools in giving rise to a current. The concentration cells belong to the third class. In concentration chains, also, the increase in electric energy with rise of temperature is proportional to the absolute temperature. It appears that the bioelectric currents possess these properties, so far as tested—*i.e.*, the potential difference increases with rise of temperature and the increase is approximately proportional to the rise; further in at least one case, that of the electric organ of the torpedo (Bernstein and Tschermak, '06), the circuit cools during the discharge, although only a few thousandths of a degree.[5]

The concentration-cell theory can be included in the metabolic rate theory advanced in this paper. For differences in metabolic rate lead to differences in the concentration of ions, which thus become the sources of potential differences. This view was presented above (p. 315). That the electromotive force is proportional to the absolute temperature need not greatly concern us, since this is also not infrequently the case where chemical reactions are obviously involved (cf. Bayliss, '15, pp. 42–43).[6]

The membrane-depolarization theory is closely related to the concentration-cell theory, in that its adherents suppose the concentration differences to arise through the presence of semipermeable membranes in living organisms. This matter is discussed by Bernstein ('02, '12), Cremer ('06), Brünings ('03), R. S. Lillie ('11, '13), and others. The chief tenets of this theory are: that the cell membranes are semipermeable membranes, which are permeable only to certain ions, chiefly, in the opinion of most authors, positively charged ions; that the cations pass through the mem-

[5] If the production of electromotive energy in organisms is an endothermic reaction, as Bernstein maintains, this may account for the lack of heat production in a stimulated nerve.

[6] It is rather amusing to note that whereas on p. 42 Bayliss severely criticises the idea that the temperature coefficient furnishes reliable information concerning the chemical or physical nature of a biological process and mentions some cases where chemical reactions are obviously involved in which the velocity is a linear function of temperature, on p. 644 he rejects the possibility that the bioelectrc currents are due to chemical reactions on the grounds that the electromotive force is proportional to the absolute temperature.

brane, leaving the anions behind; that consequently the membrane is the seat of a polarization, being positively charged externally, negatively charged internally; that upon stimulation the membrane becomes more permeable at the locus of stimulation, letting anions through; and that consequently the membrane is depolarized at the place of stimulation, becoming temporarily negative. The difficulties in the way of this conception have been pointed out by Keith Lucas ('12), and one of its former chief adherents, R. S. Lillie, has been recently strongly inclined to a chemical point of view. While the current of action is readily accounted for on the basis of the membrane-depolarization theory, it seems to us that it is more difficult to account for the current of injury and almost impossible to explain on this basis the permanent differences of potential which exist along the axes of many organisms. To get such a gradation of potential it is necessary to assume the existence of a gradation in concentration of ions along surface and interior. A difference in concentration of similarly charged ions along the axis could scarcely be brought about by the properties of the membrane; the membrane is either permeable or impermeable to a class of ions. Such concentration differences must be due to differences in the speed of production of such ions at different levels. This differential speed of production of ions with reference to level is exactly what we suggest to exist, and if it exists the assumption of membranes is unnecessary. The membrane-depolarization theory also states that the current of injury is due to the exposure of the (presumably) negatively charged interior of the tissue at the injured surface, thus producing a negativity at the cut surface. But it has long been known that the negativity due to injury is present not only at the cut surface, but also exists along the uninjured external surface, decreasing with distance from the site of injury. This is readily explained on our point of view which regards injury as a form of stimulation, accompanied by increased metabolic rate, and transmitted with a decrement to adjacent regions, as are other forms of stimulation.

So numerous are the possible sources of potential differences that it is probable that such differences in organisms are due in different instances to different factors or to a combination of factors. All that we wish to emphasize here is that certain very definite bioelectric currents, such as the current of injury, the current of action, and particularly the permanent currents which exist along the axes of the simpler organisms, are associated with metabolic differences and probably result from such metabolic differences. These metabolic differences are chiefly quantitative in nature—*i.e.,* differences of rate.

It further seems probable that these axial differences in the rate of chemical change in organisms are responsible for the phenomenon of galvanotaxis. Owing to the differences in concentration of ions at different levels, the organism is electrically polarized and hence will be expected to respond in a definite way when placed in an electric current. This matter has already been discussed by the senior author ('18).[7] The anterior ends of most of the lower and simpler animals are positively charged (int.); hence it will be expected that when placed in a current the anterior end will be directed toward the cathode. Such is the case in such organisms. In a few cases, described below, we have found that the anterior end is negatively charged (int.); such organisms orient with anterior ends toward the anode. In some flatworms, and in annelids generally, both anterior and posterior ends are positively charged (int.); such animals bend in a U-shape, with the two ends of the body directed toward the cathode. It thus appears that the internal charge, in many animals at least, determines their galvanotactic response. A suggestion to this effect was previously made by Coehn and Barratt ('05), but was incomplete, as these authors did not take cognizance of the fact, at that time but recently discovered, that there is a graded difference of potential along the axis.

One of the prevailing theories of galvanotaxis is that adopted by Loeb and discussed by him in his book on forced movements ('18). This theory assumes that the galvanotactic orientation is due to the direct action of the current on ciliary or muscular elements or on the nerve cells that control the muscles. While such action of the current may play a rôle in galvanotaxis, and may control the galvanotactic response in some animals, it seems inadequate to account for the facts in the majority of cases. The behavior of the cilia in galvanotaxis has been carefully investigated by Ludloff ('95), Pearl ('00), Wallengren ('02, '03), Statkewitsch ('05), and others, and the galvanotactic response has been assigned in most cases to the direct action of the current on the cilia. But, according to Statkewitsch ('05), *Paramecium* under optimum conditions responds to the current with very little change of the cilia from their normal behavior. Bancroft ('06) found that exposure to various salt solutions renders *Paramecium,* which is normally cathodic, anodic, and some solutions destroy the galvanotropism of this ciliate. Dale ('01) found that *Opalina,* usually described as exhibiting anodic galvanotropism, is cathodic in slightly acid media and anodic in slightly alkaline media; the same dependence of the response on the medium is likewise true of other parasitic ciliates of the frog. The behavior of the cilia is the same in either reaction. According to Terry ('06) and Bancroft ('07), *Volvox* is cathodic in the light and becomes anodic after being kept in the dark. When cathodic, the orientation is brought about by the inhibition of the flagella on the cathodal side; when anodic, by their inhibition on the anodal side. It thus appears that in *Protozoa* the galvanotactic response is determined chiefly by the internal condition, presumably ionic, of the organism, and not by the direct effect of the current on the cilia. Similar reversals have been noted by us in multicellular animals as the result of environmental conditions or prolonged exposure to the current and appear to be due to internal physiological changes and not to the action of the current on the neuro-muscular mechanism.

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[7] In this paper near the top of the second column on page 523 the statement that oligochætes in a current travel to the anode should read "travel to the cathode."

Since the inception of the ideas recorded above it has been our purpose to collect further data on the correlation between metabolic differences, electrical differences, and the galvanotactic response in animals. We hope to test in as many forms as possible the metabolic gradient along the axis, the potential differences along the axis, and the galvanotactic behavior. For the last three or four years this work has been carried on whenever opportunity has afforded. It seems desirable, owing to the time which has elapsed since the presentation of the preliminary paper (Hyman, '18), to record the principal data which have accumulated since that publication. To present these data and to point out the correlation between the three phenomena in question is the object of the present paper.

II. Methods.

The metabolic gradients have been tested by various methods, chiefly the susceptibility to various toxic solutions and to dyes, and the capacity to reduce potassium permanganate. Wherever practical, direct determinations of the respiratory rate at different levels have also been made. For a general discussion of metabolic gradients and of the susceptibility method see Child ('20) and of the permanganate method Child ('19*a*).

The potential differences have been tested by galvanometers of the D'Arsonval type, put out by the Leeds and Northrup Company. The one which has been used for most of the work is a small portable galvanometer having a resistance of 310 ohms and a sensitivity of 73 megohms (1 volt through a resistance of 73 megohms gives a swing of 1 division of the scale). During the summer of 1919, while being used by Hyman at Friday Harbor, the suspension of this instrument broke and another instrument having a spring suspension was borrowed; the sensitivity of this instrument was not recorded, but it was slightly more sensitive than ours. Part of the data on the medusæ and *Nereis* and all of the data on ctenophores was obtained with this second instrument. Non-polarizable electrodes of the zinc-zinc sulphate type were used, their tips packed with kaolin and terminating in rolls of hard filter paper, frequently changed, soaked in the medium in which the animal to be tested lives. It is necessary to renew these electrodes frequently, as they are apt to develop potential differences. These can be eliminated by running a compensating current through them as was done in part of the work. Usually the difference between the electrodes was eliminated by reversing the animal on the electrodes. In the case of sessile animals the electrodes were clamped in position and the animal placed across their tips; in motile animals the electrodes were held in the hands by means of burette clamps and placed on the animal in the desired position. It is not an easy matter to work with active animals. Usually at least two readings were taken on each individual for each position of the electrodes. The readings recorded in the table represent divisions of the scale of the galvanometer; where the differences were small, movements of the indicator of less than one division are recorded as .5. Freshly collected material was nearly always used for the galvanometer tests.

Direct current for the galvanotaxis tests was obtained from a number of cells in series or from a 110-volt direct-current generator. Metal electrodes were used, owing to the high resistance of non-polarizable electrodes. The voltage was regulated with a variable resistance shunted across the line in such a way that any voltage from zero to the maximum could be obtained simply by moving a sliding contact. When testing an animal, the slide was placed at the zero end of the resistance, the animal introduced into the testing dish, and the potential gradually increased to a strength which would evoke a definite response. Strong currents or prolonged exposure to the current often alters the galvanotactic response. The records kept were not sufficiently complete to enable us to calculate the current density in the galvanotaxis experiments with any degree of accuracy, as we did not regard such data as significant for the purpose of this investigation.

III. Sponges.

The work on sponges recorded here was done by Hyman at Woods Hole in the summer of 1919.

1. *Metabolic Gradients.*—Potassium permanganate was used for the study of the metabolic differences along the axis of two simple sponges, *Grantia* and *Leucosolenia.* Potassium permanganate is reduced by protoplasm, giving a brown stain, and the rate of appearance of this stain, as well as the depth of its color, is a rough measure of metabolic differences, since regions of higher metabolic rate reduce the permanganate more rapidly (see further Child, '19*a*). In applying this method to simple sponges it is preferable, owing to the thickness of the animal, to make a median longitudinal section and watch the appearance of the brown color along the cut surface. This was done in the case of *Grantia,* while entire individuals were tested in the case of *Leucosolenia.* In many individuals of *Leucosolenia* and *Grantia* which were tested in potassium permanganate a slight but evident gradation of staining power was noted along the axis, the oral or oscular end staining

first and more deeply, and the depth of color diminishing along the axis. The staining gradient was most marked in medium-sized individuals, practically absent in large or small individuals; the latter, however, stain more deeply than larger individuals. Child has also noted a staining gradient in potassium permanganate in a *Grantia*-like sponge at Friday Harbor.

From these observations it may be concluded that there is a metabolic gradient along the axis of these simple sponges, the oscular end having the greatest metabolic activity and this activity decreasing along the axis.

2. *The Electrical Gradient.*—Numerous tests of the potential difference along the axis of *Leucosolenia* and *Grantia* showed that in the great majority of individuals the oscular end is electronegative (galv.) to the basal end. In some cases, usually in small or very large individuals, there was no potential difference along the axis; in a few cases the potential difference was reversed, the oscular end being positive (galv.). The results are given in Table I. The potential differences are rather small.

Certain facts not presentable in the table were also noted. In large specimens there is likely to be a greater potential difference between the oscular end and the middle of the sponge than between the oscular and basal ends, indicating some degree of physiological independence of the basal region in such large specimens. There is usually no potential difference between lateral branches (*Leucosolenia*) and the main osculum—*i.e.,* the buds are also negative (galv.). Freshly cut cross-sections (*Grantia*) are markedly negative (galv.), being often negative even to the oscular end; this is, of course, merely an example of the current of injury.

In these sponges, then, is found the usual correspondence between metabolic rate and electric potential, the region of highest metabolic rate being electronegative (galv.).

IV. Hydroids.

The work on the electrical gradients of hydroids was done by Hyman at Woods Hole in the summer of 1919, with the exception of the data on *Obelia borealis,* which were obtained by Bellamy in 1918 at Friday Harbor.

1. *The Metabolic Gradient.*—The metabolic gradients of several

common hydrozoan hydroids have been studied by Child (’19*b*, ’21*b*) by means of various toxic solutions, dyes, and potassium permanganate, and have been confirmed by Hyman on the same

TABLE I.

ELECTRIC GRADIENT OF *Leucosolenia* AND *Grantia*. NEGATIVE AND POSITIVE AS IN THE GALVANOMETER.

Leucosolenia.				*Grantia.*			
No.	Oscular End.	Basal End.	Readings.	No.	Oscular End.	Basal End.	Readings.
1	−	+	.5, 0	1	−	+	.5, 1
2	−	+	1, .5	2	−	+	.5, .5
3	−	+	.5, .5	3	−	+	.5, .5
4	−	+	.5, 1	4	+	−	1
5	−	+	.5, 0	5	−	+	.5, .5
6	No potential diff.			6	−	+	.5, 1
7	−	+	.5	7	No potential diff.		
8	−	+	1, 0	8	No potential diff.		
9	−	+	2, 1	9	−	+	1
10	−	+	.5, .5, 1	10	−	+	1, .5
11	−	+	1.5, 1.5	11	−	+	.5
12	−	+	1.5, .5	12	−	+	1.5, .5
13	−	+	1, 1	13	−	+	1, .5
14	−	+	1, 1.5, 1	14	−	+	1, 1
15	−	+	1.5	15	−	+	2
16	+	−	.5, 0	16	−	+	1.5, 1
17	−	+	1	17	−	+	1, 2, 2
18	−	+	1, 1	18	−	+	1.5
19	−	+	1, 1	19	−	+	2, 1
20	+	−	1.5, 1.5	20	−	+	0, 1
21	−	+	.0, .5, 1	21	−	+	3.5, 3.5
22	−	+	1, 1	22	−	+	1
23	−	+	.5, 1.5	23	−	+	1
24	−	+	1, .5	24	−	+	1, 1
25	No potential diff.			25	−	+	1.5
26	−	+	1, .5	26	−	+	1, 2
27	−	+	.5	27	−	+	2, 1
28	−	+	1.5	28	−	+	2, 1.5
29	−	+	.5, 1.5	29	−	+	.5, 0
30	No potential diff.			30	−	+	.5
31	+	−	1, 1	31	−	+	1.5, 2
32	−	+	.5, .5	32	−	+	1, 1
33	−	+	.5, .5	33	−	+	2.5, 2.
34	−	+	1, 0, .5	34	−	+	2, 2
35	−	+	1.5, 1	35	−	+	1, 2
36	−	+	1, .5	36	−	+	1
37	−	+	1.5, 2				
38	−	+	1				
39	+	−	.5				

and other species. The gradients are the same in all of the species studied. The hydranths are more susceptible and have greater reducing power than the stems. The gradient in each hydranth proceeds from the distal to the proximal ends of the tentacles, and from the oral to the aboral end of the body of the hydranth; in stems the gradient is likewise apico-basal. In the colony as a whole and in each branch the susceptibility and reducing power are, in general, highest in the terminal hydranths and decrease basipetally among the hydranths and growing tips. In general, then, metabolism is carried on more rapidly in the distal portions of individuals, colonies, and branches and decreases basipetally.

2. *The Electrical Gradient.*—The electrical gradient of the hydroids was discovered by Mathews ('03) for *Tubularia, Pennaria,* and *Campanularia.* He found that the distal portions are electronegative (galv.) to proximal portions. This result has been confirmed by us for the same and other genera and species. The forms tested were: *Tubularia crocea, Pennaria tiarella, Obelia geniculata, Obelia borealis, Eudendrium ramosum,* and *Schizotricha (Plumularia) tenella.* The results are presented in Tables II. and III.

Tubularia crocea.—The data upon this species have been published in another connection (Hyman, '20). Hydranths are negative (galv.) to stems, distal levels of the stem are negative to proximal levels, except at the regions of branching, which are usually negative to levels immediately distal to them.

Obelia geniculata.—This species forms a small unbranched colony, 1–2 cm. in height, consisting of a simple stem bearing lateral hydranths alternately arranged, and growing by the formation of a new bud at the apical end. In making the galvanometric tests the entire colony was removed from the substratum and placed across the electrodes. The electrical differences recorded in Table II., therefore, concern the apical and basal regions of the colony; the former is almost invariably negative (galv.) to the latter.

Schizotricha tenella.—This species forms a delicate plume-like branching colony, 3–5 cm. in length. Entire colonies were placed across the electrodes; the electrical differences recorded in Table II. are, therefore, those between the apical and basal levels of the colony; the former is negative (galv.) to the latter.

Eudendrium ramosum.—The colonies of this species are large, bushy, and much branched, with hydranths considerably larger than those of the preceding two species. Most of the tests recorded in Table 2 were made upon branches of the colonies; the apical regions of such branches are negative (galv.) to the basal levels. In Nos. 3, 10, 15, and 21 a short piece was removed from the apical end of the main axis of the colony, and the potential difference between the apical and basal ends of this piece tested; the former is again negative (galv.) to the latter. In Nos. 24 and 25 the terminal zooid of the colony was compared with one of the lateral zooids, the two being connected by a strip of filter paper soaked in sea-water; the former is negative (galv.) to the latter, as was found also in *Obelia borealis*.

Table II.

Electric Gradient of *Obelia geniculata, Schizotricha tenella,* and *Eudendrium ramosum.*

Negative and positive refer to direction through the galvanometer. Readings are divisions of the scale of the galvanometer. Ap., apical; bs, basal; rd., galvanometer readings.

Obelia.				*Schizotricha.*				*Eudendrium.*			
No.	Ap.	Bs.	Rd.	No.	Ap.	Bs.	Rd.	No.	Ap.	Bs.	Rd.
1	–	+	2, 0	1	–	+	2, .5	1	–	+	.5, .5
2	–	+	.5, 1	2	–	+	1.5, 1	2	–	+	1, 1
3	–	+	1.5, 1	3	–	+	.5, .5	3	–	+	.5, 1
4	–	+	2, 1.5	4	–	+	.5, .5	4	–	+	.5, 1
5	–	+	1.5, 1	5	–	+	.5, .5	5	–	+	1.5, 1.5
6	–	+	.5, .5	6	–	+	1, .5	6	–	+	1.5, 1.5
7	–	+	1, 1	7	–	+	.5, .5	7	–	+	2, 1.5
8	No pot. diff.			8	–	+	1, 1	8	–	+	1.5, 1.5
9	–	+	.5, 1	9	–	+	1.5, 1.5	9	–	+	1.5, 1.5
10	–	+	.5, 1	10	–	+	1.5	10	–	+	1.5, 1.5
11	–	+	.5, 1	11	–	+	.5	11	–	+	1, 1
12	–	+	1, 1	12	–	+	1.5	12	–	+	.5, 1
13	–	+	1, 0	13	–	+	1.5, 1	13	–	+	.5, .5
14	–	+	1.5, 2	14	–	+	.5	14	–	+	.5, 1.5
15	–	+	.5, .5	15	–	+	1, .5	15	–	+	.5, .5
16	–	+	1, 1	16	–	+	.5	16	–	+	1, 1
17	–	+	1.5, 1.5	17	–	+	1.5, 1	17	–	+	1.5, 1.5
18	–	+	.5, 1	18	–	+	1	18	–	+	1, 1
19	–	+	1.5, 1.5	19	–	+	1.5	19	–	+	2, 2
20	–	+	.5, 1					20	–	+	.5, .5
21	–	+	.5, .5					21	–	+	1, 1.5
								22	–	+	1, 1
								23	–	+	.5, .5
								24	–	+	.5, .5
								25	–	+	2, 2

Pennaria tiarella.—This species forms large, open, branching colonies. Most of the data in the table were obtained by comparing the apical and basal ends of branches or terminal portions of the main axis; the apical end is negative to the basal end and the p.d. is greater along the distal part of the main axis than along the proximal part, and greater along the distal part of the main axis than along lateral branches. Thus No. 3 in Table III. gives the potential difference between the terminal hydranth and the stem proximal to it; Nos. 4 and 5 give the difference along lateral branches of the same colony. No. 6 is the main axis of a colony; Nos. 7 and 8 lateral branches of the same colony. Nos. 9, 10, and 11 give the potential differences along a distal 10-mm. piece, middle 10-mm. piece, and a basal 10-mm. piece, respectively, of a colony. No. 14 gives the potential difference between the apical and basal regions of a large colony; Nos. 17 and 18 of distal branches of

Table III.

Electric Gradient of *Pennaria tiarella* and *Obelia borealis*. Symbols as in Table II.

Pennaria.				*Obelia borealis.*			
No.	Ap.	Bs.	Rd.	No.	Ap.	Bs.	Rd.
1	–	+	2.5, 2	1	–	+	1, 1.5, 1, .5
2	–	+	3.5, 2	2	–	+	2, 2, 2.5, 2
3	–	+	2, 1	3	–	+	1, 1, 1, 1
4	–	+	.5, .5	4	–	+	2, 2, 2, 2
5	–	+	1, .5	5	–	+	2, 2, 2, 2
6	–	+	2.5, 2.5	6	–	+	2.5, 2, 2.5, 2
7	–	+	1, .5	7	–	+	.5, .5, .5
8	–	+	.5	8	–	+	.5, .5, .5
9	–	+	1, 1.5	9	–	+	.5, .5, .5
10	–	+	.5	10	–	+	1, .5, 1, 1
11	No potent. diff.			11	–	+	2, 2, 2, 1.5
12	–	+	.5	12	–	+	2.5, 2.5, 2, 2
13	–	+	1.5, 1.5	13	–	+	2.5, 2.5, 2.5
14	–	+	2.5, 2.5	14	–	+	2, 2, 2, 2
15	–	+	.5, 1	15	–	+	2, 2, 2, 2
16	–	+	.5, .5	16	–	+	2.5, 2.5, 2
17	–	+	.5, 1	17	–	+	1.5, 1.5, 1.5, 2
18	–	+	1, 1				
19	–	+	1.5, 1.5				
20	–	+	1.5				
21	–	+	.5, .5				
22	–	+	2.5, 3				
23	–	+	1.5, 1.5				
24	–	+	1.5, 2				
25	No potent. diff.						
26	–	+	1.5, 1				
27	–	+	.5, 0				
28	No potent. diff.						

the same colony; No. 16 of a proximal branch of the same colony; and No. 19 of the distal part of the main axis and No. 21 of the proximal part. Nos. 25, 26, and 28 are old portions of the colony with many medusæ buds; such parts show little or no apico-basal potential difference.

Obelia borealis.—This is a large much-branched colonial hydroid common at Friday Harbor. Apical levels of entire colonies are electronegative (galv.) to basal levels, as in Nos. 1 to 6 in Table III.; apical levels are negative to basal levels of branches, as in Nos. 11 to 17 in Table III.; and the main apical hydranth is negative to the apical hydranth of lateral branches, as in Nos. 7 to 10.

From these data on several species it is clear that in the colonial hydroids, in general, apical levels of the colony are electronegative (galv.) to basal levels, both as regards hydranths and stems. The electrical gradient is steeper in apical regions and slight or lacking in basal regions. These results correspond with the metabolic gradients, regions or parts of higher metabolic rate being negative to those of lower rate.

3. *Galvanotaxis.*—The slight motility of the colonial hydroids precludes the practicability of determining their galvanotactic response. Experiments were, however, tried on *Pennaria.* Branches were placed at right angles to the direction of the current. It was noted that the larger and more vigorous hydranths in such branches, usually the main or lateral apical hydranths, turned the manubrium toward the cathode during the passage of the current. When the current was reversed the manubrium was turned to the new cathode. The animals fatigue rapidly, however, and soon fail to respond; as already stated, only the largest hydranths respond. This behavior, as far as it goes, corresponds with the theory of galvanotaxis outlined above—*i.e.,* that part of the animal having the highest metabolic rate and an internal positivity is directed toward the cathode.

V. HYDROMEDUSÆ.

1. *The Metabolic Gradient.*—The gradients of these medusæ in toxic solutions, dyes, and potassium permanganate have been determined by Child ('21*b*). In all species tested the manubrium and margin, including the tentacles, are the most susceptible and

have the greatest reducing power of any parts of the animal; the subumbrellar ectoderm ranks next in activity; the exumbrellar ectoderm is the least active part of the animal. McClendon ('17) found that the greater part of the oxygen consumption of *Cassiopea* is due to the manubrium, and that the subumbrellar surface (minus the manubrium) consumes much more oxygen than the exumbrellar surface plus the mesoglœa.

2. *The Electrical Gradient.*—The potential differences tested in several species of hydrozoan medusæ correspond completely to the metabolic differences. The distal end of the manubrium is the most negative part of the animal, the margin, including the tentacles, is usually next, the subumbrellar surface next, while the exumbrellar surface is positive to all parts of the animal.

The forms tested were *Gonionemus murbachii* by Hyman at Woods Hole in 1919, *Æquoria victoria* and *Mitrocoma discoidea* by Bellamy in 1918 and by Hyman in 1920 at Friday Harbor, and *Stomotoca atra* by Hyman in 1920 at Friday Harbor. The results are given in Table IV. In *Gonionemus* the manubrium was found

TABLE IV.

ELECTRICAL DIFFERENCES BETWEEN MANUBRIUM, SUBUMBRELLA, EXUMBRELLA, AND MARGIN OF THE BELL IN FOUR SPECIES OF HYDROZOAN MEDUSÆ.

Only two points can, of course, be compared at one time. Man., manubrium; sub., subumbrella; ex., exumbrella; mar., margin; rd., galvanometer readings. Negative and positive refer to direction in the galvanometer.

Gonionemus.						*Stomotoca.*					
No.	Man.	Sub.	Ex.	Mar.	Rd.	No.	Man.	Sub.	Ex.	Mar.	Rd.
1			+	–	2.5	1	–		+		1, 1, 1.5
	–		+		11, 4				+	–	1.5, 1.5
2			+	–	5	2	–		+		4, 4, 1.5
	–		+		2, 1.5				+	–	1.5, 0
3			+	–	2, 1.5, 2	3	–		+		1.5, 1, 1
	–			+	3, 2.5				+	–	1, .5
	–	+			1.5	4	–		+		1.5, 1, 1.5
			+	–	1, 1				+	–	1, 1
4	–		+		4.5, 2.5	5	–		+		1.5, 2, 2.5
5			+	–	1, 1				+	–	1.5, 2, .5
	–		+		1, 1	6	–		+		1, 1.5, .5
6			+	–	2.5, 3				+	–	1, .5
	–		+		2, 2	7	–		+		1, 1, 2
	–			+	1, 2	8	–		+		4, 3, 2.5
7			+	–	11, 13				+	–	.5, 1
8	–		+		2, 2.5	9	–		+		1.5, 1.5
									+	–	1, 0
						10	–		+		2.5, 1.5, 1.5
									+	–	1, 1.5, 0

TABLE IV—*Continued.*

Æquorea.						*Mitrocoma.*					
1	−	+			2.5, 2, 5	1	−	+			7, 6, 9
		+		−	.5, 1.5, 2		−		+		1, 1.5, 2
		−	+		1, 2.5, 1.5			+		−	.5, 2.5, 1
2	−	+			.5, 1.5, 2	2	−	+			14, 7, 4
		−		+	5, 1.5		−		+		4, 5, 4
		−	+		4, 1, 1	3	−	+			6, 3
3	−	+			1, .5, .5			+		−	1, 2, .5
		−		+	0, 1, .5		−		+		2.5, 1, .5
		−	+		2, 2, 1	4	−	+			2, 2, .5
4	−	+			2, 1.5, .5			+		−	.5, .5, 0
		+		−	.5, .5, 1		−		+		3, 3
		−	+		1.5, 1, 1.5	5	−	+			3, 6, 7
5	−	+			2, .5, 2			+		−	1, .5, .5
		+		−	1.5, 1, 1		−		+		7, 6
		−	+		2.5, 1.5, 2	6	−	+			10, 6, 5
6	−	+			.5, 1, .5			+		−	1, 1, 1
		−		+	.5, .5, 0	7	−	+			9, 4, 2
		−	+		2, 3, 1.5		−		+		3, 3, 3
7		−		+	.5, 1			+		−	1, 1
		−	+		1, 1	8	−	+			8, 4, 3
	−	+			2, 1.5, 1.5		−		+		6, 6
8	−	+			1.5			+		−	1, 1
9	−	+			.5, .5, 0	9	−	+			4, 3, 6
		−	+		1.5, 1, 1			+		−	.5, 1.5, 1
10		+		−	.5, .5		−		+		2.5, 1
		−	+		.5, 1	10	−	+			12, 12
11	−			+	9, 4, 8		−		+		11, 6
12	−			+	4, 3, 5, 4			+		−	1, .5, 0
		−	+		7, 7, 2, 6				+	−	2, 2
13	−			+	8, 9, 3, 3	11	−			+	8, 6, 7, 5
		−	+		5, 6, 5, 3	12	−			+	4, 8
14		−	+		14, 13, 14	13	−			+	8, 12, 10, 13
		−	+		6, 6, 6, 4	14	−			+	10, 10, 9, 6
		−	+		6, 4, 4, 3	15	−			+	8, 12, 10, 13

invariably negative (galv.) to all other parts and the margin negative to the subumbrella and exumbrella. In *Stomotoca* the manubrium and the margin were invariably negative to the exumbrella, and it can be inferred from the table that the manubrium is negative to the margin; the small size and tall bell-shape of this species did not permit the testing of the subumbrellar surface. In *Æquorea* the electric conditions are somewhat dependent upon the sexual condition. This medusa has numerous radial canals which bear the gonads on their subumbrellar surfaces, as is the case in all of the Leptomedusæ; the canals are so numerous that it is impossible to avoid touching the gonads on applying the electrodes to the subumbrellar surface. In sexually ripe individuals the negativity of the subumbrellar surface is increased and it may be negative (galv.) to manubrium and margin. In Table IV. the potential difference recorded between manubrium and subumbrella is that between the distal border of the manubrium and a point on the subumbrellar surface near the base of the manubrium; the former is negative (galv.) to the latter. Comparisons between margin and subumbrella were taken on the middle of the subumbrellar surface; in four cases (1, 4, 5, 10) the margin is negative, in four cases (2, 3, 6, 7) the subumbrella is negative, due to the presence of ripe gonads. In all cases tested in *Æquorea* the exumbrella is positive (galv.) to all other parts, and the margin is positive to the manubrium. *Mitrocoma* shows the same relations as the other medusæ: manubrium negative to margin, subumbrella, and exumbrella; margin negative to subumbrella; subumbrella negative to exumbrella.

3. *Galvanotaxis.*—We were unable to find any definite galvanotactic response in the medusæ noted above, except the general tendency for the tentacles to direct themselves toward the cathode. Probably the optimum strength of current was not found. Bancroft ('04) noted a precise response to the current in the medusa *Polyorchis*. In this form the manubrium and tentacles were directed toward the cathode, thus corresponding with the expectation on the basis of our theory, as these parts of the animal are the most positive (int.) regions.

VI. CTENOPHORES.

1. *The Metabolic Gradient.*—The existence of a gradient along the plate rows of ctenophores may be inferred from the fact that the wave which passes along the plates originates usually at the aboral pole and sweeps toward the oral pole. Child ('17) also found a susceptibility gradient along the plate rows from the aboral to the oral pole. This gradient, however, is readily reversible, at least temporarily.

2. *The Electrical Gradient.*—Tests were made by Hyman in 1920 on a small spherical ctenophore common at Friday Harbor, which is probably *Pleurobrachia;* a few specimens of *Beroe* were also available. The data on *Pleurobrachia* are given in Table V. In 14 cases out of 20 the aboral pole was negative (galv.) to the oral pole; in 3 cases the oral pole was negative, and in 3 cases the

gradient reversed while it was being tested, the aboral pole being first positive and then negative in Nos. 3 and 18 and first negative and then positive in No. 7. In six tests on *Beroe* the aboral pole was negative to the oral in three cases, was negative on first readings and then became positive in two cases, and in one case there was no definite potential difference between the two poles. It was pointed out above that physiological reversal of the wave in the plate rows is common.

3. *Galvanotaxis.*—No definite response to current was found in *Pleurobrachia,* nor to our knowledge has the matter been tested by others.

VII. Flatworms.

Definite tests of forms belonging to this group were made by Hyman on *Planaria maculata* at Woods Hole in 1919 and on an unidentified polyclad turbellarian at Friday Harbor in 1920.

1. *The Metabolic Gradient.*—This has been studied by members of this laboratory for several species. The anterior end has the highest metabolic rate and this rate decreases along the axis to the level of the main fission plane, beyond which it increases again. It has also been found that the carbon dioxide production (Robbins and Child, '20) and the oxygen consumption (Hyman, '23) are higher for anterior than for posterior levels of the first zooid. In *Planaria maculata,* tested with potassium cyanide, the following condition was found: The disintegration begins at the anterior end and progresses posteriorly along the margins of the head and the ventral surface; it then begins at the posterior end and progresses forwards; the two waves of disintegration meet at about the middle of the worm. There is thus in this species a "double" gradient, from the two ends of the body toward the middle. The high susceptibility of the posterior end is probably due largely to the use of this end as an adhesive organ, less to the presence of a second zooid there. Very small worms possessed the same susceptibility gradient as large worms. The susceptibility gradient of the polyclad was not tested, owing to scarcity of material.

2. *The Electrical Gradient.*—The data on the metabolic gradient would lead us to expect that the anterior end of *Planaria maculata* will be negative (galv.) to other levels of the body. This was

found to be the case as shown in Table V. in the great majority of cases. The small size of the worm did not permit the testing of more specific regions of the body; it is possible merely to place the electrodes on general anterior and posterior levels. The lack of potential difference in Nos. 2, 3, 8, and 17 is probably due to the circumstance that in these individuals the susceptibility of the posterior end was practically equal to that of the anterior end.

Table V.

Electrical Differences Between Aboral and Oral Poles of the Ctenophore *Pleurobrachia*, Anterior and Posterior Levels of *Planaria maculata*, and Anterior, Middle, and Posterior Levels and Margins of a Polyclad.

Ab., aboral; or., oral; ant., anterior; mid., middle; post., posterior; mar., margin; rd., galvanometer readings. Reversed, reversed during test. Negative and positive refer to direction in the galvanometer.

Pleurobrachia.				*Planaria.*				*Polyclad.*					
No.	Ab.	Or.	Rd.	No.	Ant.	Post.	Rd.	No.	Ant.	Mid.	Post.	Mar.	Rd.
1	−	+	1, .5, .5	1	−	+	1, .5	1	−	+			.5, 1, 2, 1
2	−	+	1.5, 1, 1	2	no p. d.					+	−		1, 1.5, 1.5
3	reversed			3	no p. d.					+		−	4, 2.5, 2
4	−	+	0, .5, 0, .5	4	−	+	.5, .5	2	−	+			1.5, .5, 2, 3
5	−	+	.5, .5, .5	5	−	+	1, 1			+	−		1.5, 2, 2
6	−	+	.5, .5, .5	6	−	+	.5, .5			+		−	2, 2, 2.5
7	reversed			7	−	+	1, .5	3	−	+			2.5, 2, 2
8	−	+	.5, .5, .5	8	no p. d.					−	+		.5, .5, 1
9	−	+	.5, .5, 1.5	9	−	+	.5, 0			+		−	.5, .5, .5
10	−	+	.5, 1.5, .5	10	−	+	1.5, 1	4	−	+			1.5, 3, 2
11	+	−	1, .5, 0	11	+	−	1, 1			reversed			
12	−	+	.5, 0, .5	12	−	+	1, 1			+		−	.5, 0, .5
13	−	+	.5, .5, .5	13	indefinite			5	−	+			1, 1.5, 1
14	−	+	.5, .5, .5	14	−	+	1, 1.5			+	−		1, .5, 1
15	−	+	1, .5, .5	15	−	+	.5, .5	6	−	+			1, 2, 1.5
16	+	−	1, .5, .5	16	−	+	1, .5			−	+		1.5, 1,.5
17	+	−	1.5, 1.5, 1	17	no p. d.			7	−	+			.5, .5
18	reversed			18	−	+	.5, .5						
19	−	+	.5, 2.5, .5	19	−	+	1, 1						
20	−	+	1.5, .5, 1	20	−	+	1, 1						

Of the polyclad mentioned above only seven specimens were available. In all of these the anterior end was negative (galv.) to the middle; in most of them the posterior end was also negative to the middle (this reversed while being tested in No. 4), and in the four cases in which the matter was tested the margins were negative to the middle (Table V.).

3. *Galvanotaxis.*—When placed in the electric current, *Planaria maculata* orients in a very definite manner. The animal curves into a U-form, lying upon one side, anterior and posterior ends and likewise ventral surface being directed toward the cathode, middle and dorsal surface directed toward the anode. Twenty specimens were tested, all of which exhibited essentially the same behavior. The cathodic orientation of the anterior end is more marked than that of the posterior end, and the former is generally in advance of the latter. The animals often assume the posture in question at intervals, hold it for a short time, and between such postures wander about the pan without showing any definite orientation to the current; in other cases the posture was held as long as the current passed. It is evident that there is a remarkable correspondence between the galvanotactic orientation of this species and the double metabolic gradient described above. The electrical gradient, in as far as the data go, also corresponds.

The orientation of the polyclad in the current was very similar to that of *Planaria maculata*. After some preliminary contortions, all of the specimens tested turned their anterior ends toward the cathode, often assuming a U-shaped posture. If the animal is facing the cathode when the current is made, it remains in that position, and may curve the tail under the body so that the posterior end of the tail faces the cathode. If the animal is facing the anode when the current is made, it curves its anterior end under the body so that head, tail, and ventral surface face the cathode. If placed at right angles to the current, the head turns to face the cathode. The animals did not usually travel in the current; the margins of the body were kept in constant undulating movements; the animals were obviously much more stimulated when facing the anode than when facing the cathode.

VIII. Annelids.

1. *The Metabolic Gradient.*—The gradient of the chætopod annelids has been described by Hyman ('16) and Hyman and Galigher ('21). The gradient is of the double type, the metabolic rate being high at anterior and posterior ends and decreasing from both ends to the middle region. Tests of the oxygen consumption of pieces from different levels show that posterior pieces consume

the most oxygen, anterior pieces next, and middle pieces least (Hyman and Galigher, '21).

2. *The Electrical Gradient.*—It was found by Morgan and Dimon ('04) that in two species of earthworm, *Lumbricus terrestris* and *Helodrilus* (*Allolobophora*) *fœtida,* the anterior and posterior ends are, in general, electronegative (galv.) to the middle. This result was verified by Bellamy on *Helodrilus caliginosus.* Other oligochætes have not as yet been tested.

Among the polychætes experiments have been performed chiefly on *Nereis, Nereis virens* on the Atlantic coast by Hyman in 1919 and *Nereis virens* and *Nereis vexillosa* at Friday Harbor by Bellamy in 1918 and Hyman in 1920. It was found that the electrical conditions are highly dependent on the freshness of the animals. It appears that in both species the two ends are negative (galv.) to the middle in very freshly collected animals, but the data are not as yet conclusive. It is certain, however, that when the animals have been kept in the laboratory, if only for a few hours, the gradient is reversed, and the anterior end is always and the posterior end usually positive (galv.) to the middle. Table VI. gives data on the electrical conditions in laboratory animals of both species.

3. *Galvanotaxis.*—The galvanotactic response of oligochætes and leeches was tested by Blasius and Schweizer ('93) for the leech, *Branchiobdella,* and *Lumbricus,* and by Nagel ('95) for the leech, *Lumbricus,* and *Tubifex.* These authors found that all of these forms are definitely cathodic, turning their anterior ends toward the cathode, and usually crawling toward the cathode. The matter was investigated in more detail by Moore and Kellogg ('18) for *Lumbricus terrestris.* They noted that this species bends into a U-shape when placed in the current with anterior and posterior ends directed toward the cathode, middle toward the anode, and crawls toward the cathode maintaining this posture. Bellamy found the same behavior in *Helodrilus caliginosus.* Hyman has tested the behavior in the current of *Dero limosa* and *Lumbriculus inconstans.* Both are markedly cathodic. The former instantaneously places itself with longitudinal axis parallel to the current, head to the cathode, and crawls rapidly to the cathode. *Lumbriculus* assumes the same posture as *Dero,* except that the posterior

end is generally curved toward the cathode, the attitude being, therefore, similar to that of the earthworms.

In *Nereis* the galvanotactic behavior is, like the electrical gradient, dependent upon the freshness of the individuals. It appears, although this has not been conclusively established, that very freshly collected animals are cathodic; they bend their bodies into a U-shape, with anterior and posterior ends directed toward the cathode. Animals which have been kept in the laboratory (and probably also sexually ripe animals) are anodic. They assume the U-position, but with anterior and posterior ends directed toward the anode, and middle toward the cathode. All of the individuals recorded in Table VI., of which the anterior ends are negatively charged (int.), were found to be anodic.

Table VI.

Electrical Gradient of *Nereis virens* and *Nereis vexillosa* after being kept in the Laboratory.

Ant., anterior; mid., middle; post., posterior; rd., readings on galvanometer. Negative and positive refer to direction through the galvanometer.

Nereis vexillosa.					*Nereis virens.*				
No.	Ant.	Mid.	Post.	Rd.	No.	Ant.	Mid.	Post.	Rd.
1	+	−		5, 6	1	+	−		7, 5
		+	−	10, 8, 13	2	+	−		3, 3
2	+	−		15, 9, 13, 12	3	+	−		6, 6, 10
		−	+	7, 16, 16			−	+	1, 1
	+	−		31, 25, 14, 13	4	+	−		2, 6
		−	+	5, 0, 3	5	+	−		6, 6
		−	+	9, 7			+	−	1, 2
	+	−		5, 5, 6, 7	6	+	−		3, 3
		−	+	4, 5			+	−	5, 7
3	+	−		4	7	+	−		4, 7
		−	+	7, 4, 5, 5, 6	8	+		−	10, 9
4	+	−		12, 8, 7		+	−		6
		−	+	2, 2	9	+	−		4, 3
5	+	−		6, 9, 3	10	+	−		3, 5
		−	+	2, 2	11	+	−		2, 3, 6
6	+	−		5, 6			−	+	8, 8
		−	+	2, 1, 3					
7	+	−		5, 5					
8	+	−		4, 3					
9	+	−		8, 7, 4, 5					
		+	−	4, 2, 2					
10	+	−		4					
		+	−	4, 7					
11	+	−		5, 4					
		−	+	2					
12	+	−		8, 8, 6					
		+	−	5, 2					

There is thus in oligochætes a remarkable correspondence between the double gradient, the electrical gradient, and galvanotaxis. The U-attitude assumed in the current is consistent with the double respiratory gradient and double electrical gradient found in these forms. Particularly striking is the cathodic orientation of those forms of which the anterior end is positively charged (int.) and the anodic orientation of those of which the anterior end is negatively charged (int.).

IX. TADPOLES.

Although some fragmentary data have been obtained on other vertebrate young, we shall confine our discussion for the present to frog tadpoles.

1. *The Metabolic Gradient.*—This has not been determined for tadpoles, but from the condition in earlier stages of the frog (Bellamy, '19) and in other vertebrate embryos it is probable that the posterior end has the highest metabolic rate of any part.

2. *The Electrical Gradient.*—Hyde ('04) tested the potential differences along the axis of toad (?) tadpoles and states that there is a permanent difference along the axis "in a direction from the tail to the head of the embryo."[8] The meaning of this is not very clear, but from the usage throughout the paper it appears that Hyde means that the tail is negative (galv.) to the head. This was also found to be the case by Bellamy in frog tadpoles. The data are given in Table VII. In nearly all cases the head was found to be positive (galv.) to body and tail.

3. *Galvanotaxis.*—It has been known since 1885, when Hermann discovered the fact, that frog tadpoles are anodic—*i.e.,* when placed in a current, they turn their anterior ends toward the anode, tails to the cathode, bodies in line with the current. This statement we have verified. This orientation in the current corresponds completely with the electrical differences of potential found in the

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[8] In a previous paper (Hyman, '18) it was erroneously stated that Miss Hyde had determined that the anterior end of vertebrate embryos is negative to the posterior end. She found a permanent difference of potential along one axis of the blastoderm but was unable to determine the direction of the current with reference to the future embryo, owing to the young stages with which she worked, except in the case of the tadpoles, where her meaning is not clear.

same lot of tadpoles. No doubt the age of the tadpoles is a factor in the response, as both the electrical gradient and the galvanotactic response were found by Bellamy to be irregular in older tadpoles.

Table VII.

Electrical Gradient of Frog Tadpoles.

Negative and positive refer to direction of the current through the galvanometer.

No.	Head.	Body.	Tail.	Readings.
1	−		+	12
	+		−	50, 35
2	+		−	13, 12, 2, 3
	+		−	29, 9, 2
	+		−	25, 11, 9
	+		−	25
3	−		+	7, 7, 7, 9
	+		−	5, 6, 5, 6
4	+		−	7, 3, 4, 3
	+		−	10, 10
5	+		−	3, 3, 2, 2
	+		−	11, 10, 12
6	+		−	6, 10, 10, 8, 9
	+	−		6, 7, 12, 8, 7
		+	−	7, 7, 8
		+	−	6, 6, 7
7	+		−	3, 7, 6, 6, 5, 6, 5, 5
	+		−	8, 8, 8, 11, 9
	+	−		15, 11, 13, 12

X. Summary.

1. The idea is advanced that differences of potential in organisms, particularly the permanent differences which exist along the main axis of animals, are due to differences in metabolic rate at different regions, the region of highest metabolic rate being the most negative in the external circuit, most positive in the internal circuit. It is further suggested that internal potential differences account for the galvanotactic response, in many animals at least. Data are presented in various groups of animals to show the correlation between metabolic differences, electrical differences, and galvanotactic orientation.

2. In the sponges, *Leucoselenia* and *Grantia,* the oscular end has usually a higher metabolic rate than, and is electronegative (galv.) to, the basal end.

3. In the colonial hydroids tested the apical hydranths and levels

of colonies have a higher metabolic rate than, and are electronegative (galv.) to, basal hydranths and levels. In the one species tested the apical end of the hydranth is cathodic.

4. In the hydromedusæ tested the metabolic rate and electronegativity (galv.) are greatest in the manubrium, next in the tentaculate margin, next in the subumbrellar surface, and least in the exumbrella. So far as tested, manubrium and tentacles are cathodic.

5. In the ctenophore, *Pleurobrachia*, the metabolic rate and the electronegativity (galv.) are highest at the aboral pole and decrease toward the oral pole. No galvanotactic response was obtained.

6. In *Planaria maculata* anterior and posterior ends have a higher metabolic rate than the middle; the anterior end is electronegative (galv.) to posterior levels. In a current a U-shape is assumed with anterior and posterior ends directed toward the cathode, middle toward the anode. In a polyclad worm anterior and posterior ends and margins were found to be electronegative (galv.) to the middle regions; the galvanotactic response was similar to that of *Planaria*.

7. In annelids anterior and posterior ends have a higher metabolic rate than the middle and are electronegative (galv.) to it. In *Nereis,* after being kept in the laboratory, anterior and posterior ends are electropositive (galv.) to the middle. When placed in the current, all oligochætes tested turn their anterior ends toward the cathode, and the larger forms also bend their posterior ends toward the cathode, the body assuming a U-shaped posture. *Nereis,* in which the two ends are positive (galv.), assumes the same posture, but with the ends facing the anode.

8. In frog tadpoles the posterior end has the highest metabolic rate and is electronegative (galv.) to anterior levels. In a current they orient with posterior end directed toward the cathode, anterior end toward the anode.

9. It thus appears that so far as our tests have proceeded that regions of higher metabolic rate are externally negative, internally positive, to regions of lower metabolic rate, and that when placed in a current animals direct those parts positively (int.) charged toward the cathode and those parts negatively (int.) charged toward the anode.

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THE ACCELERATION OF THE RATE OF CELL DIVISION.[1]

A. RICHARDS.

Cell division is a property of all animal and all plant cells upon which depends the ability of organisms to carry out progressive differentiation. While individual cells may grow and become specialized, a limit to the differentiation of the body cells is reached if division is lacking, a fact which finds illustration in the work on tissue cultures where the cells, though they may be maintained alive for protracted periods, do not greatly change in character, particularly if the mitotic ratio is low.

Differentiation in an organism consists of processes of several types. In an embryo the first differentiation processes must take place among the cells as units. Subsequently other processes begin within the cells themselves which result, for example, in the formation of contractile fibers in muscle cells, or in other well-known changes in cells of other kinds. These later processes do not usually begin, however, until the rate of division becomes differential. Conklin has defined differential division as follows: "Cell division is typical and non-differential when it occurs at regular intervals or at the same time in cells of the same generation (rhythmical) when successive divisions are at right angles (alternating), when daughter cells are of similar size (equal), and are composed of similar materials (homogeneous). Divisions are differential when they depart from these typical conditions in one or more respects, becoming non-rhythmical, non-alternating, unequal, or heterogeneous."

In an organism, then, the progressive differentiation of the cells must be thought of as cumulative, since in each cell generation the progress begins where it left off in the proceeding. The problem of the organization of the early embryo is largely one of the cumulative differentiation of the individual cells, of their increase in number, and in the complexity of the relationships which they assume to each other. Later the intracellular processes of specialization assume a larger importance. The rapidity of organization, therefore, is directly dependent upon the rate of division of the constituent cells of the embryo.

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[1] Studies from the Zoölogical Laboratory of the University of Oklahoma, Second Series, No. 24.

Differentiation, of course, is not synchronous with the division of any particular cells. In fact, growth, and the processes which accompany it, alternate in time with the successive mitoses. Laughlin obtained evidence of this fact in his investigations on the duration of the mitoses in the onion root tips. He pointed out that "in a growing tissue, so far as the individual cell is concerned, there is a definite alternation between permanent increase in bulk and mitosis. Indeed, if bulk increase is largely anabolic and cell division catabolic, as is most probably the case, then opposing activities can not synchronize in the same cell each as a dominant factor of activity. But synchronization of the same activities among many neighboring cells is a different matter. This exists and its degree determines the character of the pulsation observed in rate of growth in actively growing tissues." From the standpoint of a careful analysis, the processes which we usually speak of as growth are to be distinguished among themselves; mitosis, increase in bulk, and differentiation are all involved. Mitosis and increases in bulk can not usually take place in one cell at the same time. Their independence in this relation, however, does not mean that the three processes are not dependent on each other, for, of course, they must maintain their proper balance or the organism can not undergo further development.

That differential rates of division in the various organs and tissues of an embryo exist can easily be shown. A casual inspection of sections of any embryo will show that not all regions exhibit cells in division with equal frequency. The mitotic ratio (by which is meant the number of cells in some stage of mitosis that can be counted in one hundred cells of the type in question) varies within rather wide limits. From some investigations which have been going on in this laboratory it appears that the ratio for early chick embryos in their entirety varies from a fraction of 1 per cent. to about 5 per cent. In any embryo many division figures may be found in some sections, while in others almost none occur. Stockard has made good use of the conception of differential rate of division in his recent paper which elaborates a hypothesis to account for the production of monsters upon this basis. Numerous investigators have reasoned that mitoses occur periodically, and that the duration of the periods may be influenced by various external factors. But these investigations leave unanswered many questions and raise many problems as to the rate of division that merit closer study. What is the normal rate of division for various tissues? What is the duration of each stage of mitosis? Do mitotic cycles go in waves? To what extent do all the cells of a given tissue divide simultaneously? If divisions are not synchronous, what changes in the mitotic ratio occur? What factors influence the rate of cell division? What are the limits of its modifiability?

One of the earliest attempts to study the rate of cell division resulted in the formation of the well-known "Balfour's law of cleavage." This law states in effect that the rate of cleavage is inversely proportional to the amount of deutoplasm that is contained within the dividing cell. Wilson's criticism of this law ("The Cell," p. 366) is all that is necessary to show that the formulation of it made no real addition to our knowledge of the subject. He said: "The entire inadequacy of this view has been demonstrated by a long series of precise studies on cell lineage, which show that while the large deutoplasm bearing cells often do divide more slowly than the smaller protoplasmic ones, the reverse is often the case, while remarkable differences in the rhythm of division are often observed in cells which do not appreciably differ in metaplasmic content. All the evidence indicates that rhythm of divisions is at bottom determined by factors of a very complex character which can not be disentangled from those which control growth in general. Lillie ('95, '99) points out the very interesting fact, determined through an analysis of the cell lineage of molluscs and annelids, that the rate of cleavage shows a direct relation to the period at which the products become functional. Thus in *Unio* the more rapid cleavage of a certain large cell ("D. 2"), formed at the fourth cleavage, is obviously correlated with the early formation of the shell gland to which it gives rise, while the relatively slow rate of division of the first ectomere quartet is correlated with reduction of the pretrochal region. The prospective character shown here will be found to apply also to other characters of cleavage."

Throughout the literature of embryology one finds an occasional reference or allusion to the actual time that is consumed in the various early cleavages, but many of these are rather incidental and lack the character of systematic studies of the point. Of those in which information of real value is given, Conklin's notations on the rate of cleavage in *Cynthia* may serve as an example. The eggs of *Cynthia* were fertilized in a typical case at 5 o'clock P.M. and the development carefully followed through. The actual duration of the various stages was as follows:

Fertilization at 5 P.M. to first cleavage (1–2 cell)	40 min.
First to second cleavage (2–4 cell)	30 min.
Second to third cleavage (4–8 cell)	20 min.
Third to fourth cleavage (8–16 cell)	20 min.
Fourth to fifth cleavage (16–32 cell)	20 min.
Fifth to sixth cleavage (36–64 cell)	20 min.
Sixth to seventh cleavage (65–112 cell)	20 min.
Seventh to eighth cleavage (112–218 cell)	20 min.
Eighth to young tadpole.	2 hours.

Or, summarizing his observations, the period from fertilization to first cleavage is 40 minutes, from the first cleavage to the beginning of gastrulation 140 minutes, and again 140 minutes to the fully formed tadpole stage. This is rather rapid development, although in *Molgula*, another ascidian, the entire period is only two thirds as long.

The writer in 1914 recorded the average duration of the early cleavages of *Planorbis* eggs under average temperature conditions of the water of about 16–20° C. The durations of the periods required for the completions of the first, second, third, fourth, and fifth cleavages were, respectively, 1½ hours, 1¼ hours, 1¼ hours, 1¼ hours, and 1 hour.

For *Haminea virescens*, a typical case, recorded in connection with the experiments reported herein, gave the following figures for the durations of the first five successive cleavages: 85 minutes, 51 minutes, 59 minutes, 50 minutes, and 40 minutes.

In all these cases the actual duration of time differs, but the first cleavage is longer than those which follow it.

Somatic tissue cells have been followed through their divisions by a few investigators who have observed them either in growing mesenchyme of a tadpole tail (Clarke) or in tissue cultures (Lambert, Lambert and Hanes, Levi, and Lewis and Lewis). Clarke observed that the prophase and metaphase together lasted about 1 hour and 15 minutes, the anaphase 4 minutes, and the telophase 3 to 4 minutes (although complete separation of the cytoplasm followed much more slowly). Lambert and Hanes found in tissue culture that at 37° C. connective tissue cells of the cat divide in 15 to 30 minutes, while mitosis in similar cells of the rat last from 25 to 45 minutes. Levi studied tissue cultures from chick embryos and arrived at the following range of variation for the phases of mitosis therein: Prophase and metaphase together, 20 to 30 minutes, of which 8 to 13 minutes, according to further observations of his, were consumed by the metaphase; anaphases usually range from 3 to 7 minutes; telophases last from 1 to 10 minutes, although the great majority were from 3 to 6 minutes in duration. Lewis and Lewis made careful observations on the duration of various phases of mitosis in mesenchyme cells in tissue cultures and concluded that the time ranges for the various stages are as follows: prophase, 30 to 60 minutes; metaphase, 2 to 10 minutes; anaphase, 2 to 3 minutes; telophase, 3 to 12 minutes; and the reconstruction period, 30 to 180 minutes.

Laughlin has made the most extensive study of the duration of mitotic stages in dividing root tip cells of the onion. He employed

Stage Number.	Stage Description.	Average Duration, in Minutes.			
		At 10° C.		At 20° C.	At 30° C.
	Resting	194.92 min.		159.57	33.26 min.
1	Early prophase	52.2550	88.2693	59.2592	51.4147
2	Early prophase	22.1064		8.2376	1.6673
3	Mid prophase (spireme)	9.2036		3.1094	1.1776
4	Late prophase	4.7043		3.3943	1.2301
5	Metaphase	1.3859		.9849	.3212
6	Early anaphase	.6539	2.2109	.7651	.3264
7	Mid anaphase	.7222		.6267	.2273
8	Late anaphase	1.8348		1.1233	.4314
9	Early telophase (di-spireme)	2.0398	4.675	1.5303	.7054
10	Late telophase	2.6352		2.3443	.7579
	Totals	292.52		240.97	91.56

a detailed statistical treatment of a total of 55,000 cell counts, divided the mitotic process into ten stages whose limits were artificially defined, and determined the actual time of each stage at 10°, 20°, and 30° C. For the period of rapid elongation of the root tips the values for each stage are shown in the tabulated résumé.

Laughlin's observations upon the periodic character of mitosis and its rhythms have already been referred to.

The factors which govern cell division are not as yet clearly understood. In a matter of such vital character as mitosis and of such complexity it is not to be expected that clear conceptions will be easily forthcoming. One method by which an analysis may be attempted is that of modifying the process by various means. At the present time little in the way of reliable data is at hand to indicate to what extent or by what factors the rate of division may be modified.

There are many factors that will act to decrease the rate; in fact, any condition which is unfavorable to the growth and well-being of the organism, or which lowers its general metabolic activity, results in a decreased rate of division in tissues where multiplications are taking place. Some of these factors are definite and measurable in their results, as decreased temperature, while others are intangible and seem to affect the vitality of the entire organism rather than the mitotic mechanism. Because of this latter set of factors it is not profitable to attempt here a study of the causes of a lowered rate of multiplication.

On the other hand, the factors which can cause an increase in the rate of division are few, and must in the nature of the case work their effects directly upon the mechanism of mitosis. It is within the bounds of hope that their study will throw light upon this mechanism as well as upon the matter of differentiation and organization. Since little attention has been given to this matter by cytologists and experimental embryologists, it seems desirable to list the means by which increased division rates have been obtained and to point out at some length the nature of their effects.

The list of agencies by which acceleration of the rate of division may be accomplished is not a long one. One might infer that any influence which will stimulate growth would also accelerate the division rate, but the evidence is not clear that such is always the case. For instance, Yung in 1881 exposed the eggs of *Limnæa stagnalis* to light of various wave lengths and found that the hatching periods varied from 17 days in the case of violet to 36 days in that of red; it would seem that the light must have accelerated the division rate, but since the data were not taken with this point in view, further evidence is necessary. The list of agencies in which the evidence is definite includes heat, x-rays, radium, thyroid secretion, supra-renal extract, alcohol, dibasic potassium phosphate, potassium sulphate, potassium bromide, oxygen, sodium hydroxide, and pilocarpine hydrochlorate.

That the rate of development of an organism depends upon temperature is a matter of common information. The development is retarded at low temperatures and increased as the temperature rises toward a maximum (above which, however, the rate almost instantly falls off until death results). The first careful inquiry into these matters was made by Oscar Hertwig ('96 and '98) upon the eggs of *Rana fusca*. Since Hertwig many investigators have studied the effects of temperature. The correlation between the increase in the rate of division and the rise in temperature has suggested that van't Hoff's law may apply to the mitotic processes. Mitosis, however, may not be looked upon as a simple reaction which would respond directly to temperature rises, so the results, as might be expected, have not been entirely uniform upon this point. Laughlin investigated the division rates at 10°, 20°, and 30° in his studies on the onion root tip and determined the values of Q_{10} (the velocity increase at a given temperature compared with the velocity of the same stage at 10° C. lower). He says: "From the Q_{10} values derived from these comparisons it is found that each mitotic stage presents characteristic velocity reactions to temperature increments. These reaction values approximate van't Hoff's expectations, thus indicating that most probably the repertoire of activities constituting each stage is composed of the actions and interactions of those much more elementary physical and chemical forces which measured in more isolated relations have been shown to react in this same velocity fashion."

The acceleration of the cleavage rates of eggs, due to their exposure under certain conditions to x-rays and radium rays, are perhaps the most marked which any of the stimulating agents are able to bring about. The effect of the radium rays upon division rates have been studied by Hertwig, Packard, and others. Packard exposed *Arbacia* eggs to radium rays and noted that "A short radiation brought about a stimulation; while a longer one produced a retard. Between these two limits there was a strength of radiation which produced no noticeable effect—*i.e.,* the initial acceleration was overcome by a subsequent retard." He argues that these results were brought about because the radiation thus effects the enzymes of the cells. These conclusions are confirmatory to a preliminary experiment of Lazarus-Barlow and Bonney and to subsequent extensive research by Lazarus-Barlow and Beckton. Since these two latter papers appeared in a publication not generally accessible to zoölogists in this country, it seems worth while to quote the conclusions in full:

"If radium act on ova of *Ascaris megalocephala* in the resting stage in quantities of the order 5×10^{-7} mgr. and for a continuous period of about 30 hours at 0° C., cellular division subsequently proceeds at an accelerated rate.

"Greater quantities than the above or more prolonged exposures progressively retard the rate of division.

"These effects are brought about by the action of alpha, beta, and gamma rays acting together.

"Beta and gamma rays alone (alpha rays being excluded) act similarly.

"The action of the alpha rays appears to be about one hundred times as great as the action of the beta rays."

Packard, Lazarus-Barlow and Beckton, and Mottram all agree that the acceleration is greater if the exposures are made in the dividing stages of nuclei rather than during resting periods. Mottram concludes "that the animal cell, as exemplified by the ova of *Ascaris,* is at least eight times as vulnerable to the beta plus gamma rays of radium in the dividing as in the resting stage of its nucleus; and, further, that this increased vulnerability during division concerns the metaphase." This general conclusion that eggs are more susceptible during the height of the division stages is one that is familiar to experimental embryologists, having been demonstrated with many eggs and many agencies.

Bohn found that an exposure to radium of forty minutes accelerated segmentation in eggs of the sea urchin, although a longer exposure retarded it.

The use of x-rays agrees in most respects with that of radium. Gilman and Baetjer exposed hen's eggs for ten minutes daily to x-rays. During the first thirty-six hours the development was accelerated. Then there followed a retardation during which the development was greatly altered as well as checked. Comparable results were obtained by these same investigators working on the eggs of *Amblystoma*. Exposures of fifteen minutes daily first produced a period of acceleration which lasted up to ten days in some embryos, but at the end of the fourth day abnormalities began to manifest themselves.

While investigating the effect of x-radiation on *Planorbis* eggs the writer found convincing evidence of their ability to accelerate cleavage. The eggs normally require from fifty-five minutes to two hours to complete a division (up to the 24-cell stage). The first effect of exposure of not to exceed ten minutes, if made during the formation of the mitotic spindle, is to accelerate the division of the egg. Even a very short stimulation will produce this phase of acceleration, which is then followed by a phase of depression; the end result is to retard greatly the development of the egg. The acceleration of the rate of cleavage in *Planorbis* as a result of exposure to x-radiation is the most extreme response of this character with which the writer is acquainted. "This effect was first obtained after eggs had been exposed ten minutes, when it was noticed that divisions had actually been completed in cells where only a spindle was to be seen at the time the exposure began—that is, during an exposure of ten minutes there had been accomplished a complete process which never under normal conditions had been observed in this form to occur in much less than an hour. I have repeated this observation from January to June on many experiments and have obtained the result without variation. Whenever an egg of *Planorbis* in any cleavage up to the sixth, farther than which it is not practical to carry on observations on the living egg, is exposed to x-rays any mitosis which may have been started is hastened to completion, and in almost every case that state has been reached by the time the egg can be taken from under the tube and examined under the microscope." During the depression phase following exposure of this egg a second stimulation may be brought about by reëxposure, but the extent of the acceleration is less and the second depression follows more rapidly.

A later experiment of slightly different character, but bearing, I think, upon this general problem, may be noted here. The writer exposed pepsin and diastase to x-rays and found that a short radiation accelerated the activity of both these preparations of enzymes, that a longer exposure inhibited the activity, and that between these two strengths there lies a point at which radiation is non-effective. Exactly similar results were obtained by the writer and Miss Woodward upon exposing the cell extractive of Echinoderm eggs, "fertilizin," to x-radiation. The parallel between the behavior of these enzymes and extractives and that of mitotic processes under the influence of radiation is probably not without its significance.

Certain substances derived from internal glands are thought to favor growth, chief among them being thyroid constituents. It might be presumed from this effect that these substances would also stimulate cell division. Nowikoff, Shumway, and also Buddington and Harvey have investigated the effect of thyroid upon ciliates and have arrived at essentially the same conclusion. Shumway added a small amount of thyroid emulsion to the hay infusion in which *Paramœcium aurelia* were growing and a sharply marked increase in the division rate resulted. He reports as follows: "Thyroid substance fed to *Paramœcium aurelia* or *caudatum*, either as an emulsion of raw thyroids or as a suspension of the commercial powder, produces a constant and significant increase of 65 per cent. in the rate of division over that observed in the common laboratory hay-medium infusion. The thyroid is the only one of the internally secreting glands that produces this effect. Boiling the thyroid produced no change in the reaction. Iodothyrin and iodine fail to produce the thyroid effect. *Paramœcia* after prolonged thyroid treatment revert to the normal division rate when returned to the control medium. The life-history curves of the thyroid-treated lines show the same depression periods at the same time intervals as the control lines, and thyroid produces the greatest acceleration of the division rate when the control line is dividing most rapidly." In one experiment, however, the use of thymus, adrenal, and pituitary substances also gave an acceleration of the rate of division, but other experiments did not confirm these data.

In experiments which are somewhat similar to those of Shumway, Miss Chambers obtained results which indicate that the division rate of *Paramecia* was increased by the feeding of ground yeast, of pituitary preparation, and especially of suprarenal solution. In the latter case "these results are more constant than those obtained by feeding pituitary solution. There is a slight but decided increase over the control lines."

Paramœcium has been the subject of a number of experiments of this sort, for it has responded by alterations of its division rate to several kinds of stimuli. Calkins and Lieb found that one part of alcohol in about 2,500 parts of culture medium acted continuously to accelerate the division rate. Woodruff has shown that the effect of small amounts of alcohol on *Paramœcium* and *Stylonychia* as well is to produce a much more rapid rate of division in the experiment than in the control during the first month of the work, but after that the rate decreased as compared with the control, and this is followed by fluctuations both above and below the control. Woodruff had earlier shown that dibasic potassium phosphate (K_2HPO_4) caused an acceleration of the rate of division during the early part of the cycle of an *Oxytricha fallax* culture, and a retardation during the latter part. Potassium sulphate (K_2SO_4) and potassium bromide (KBr) in *n*/100 solutions likewise caused slight acceleration. The stimulating effect of the treatment gradually wears off and the agents become depressants, but an increased dose will again cause a stimulation. The temporary effect of alcohol upon the rate of division of *Paramœcium* reminds the writer of his own observations on the effect of x-rays upon the rate of cleavage of *Planorbis* eggs.

The effect of oxygen upon developing eggs has been studied by a number of investigators with various problems in mind. In one case results were obtained which allow conclusions to be drawn as to the effect upon the rate of division. Godlewski found that an atmosphere of pure oxygen accelerates the development of the eggs of *Rana temporaria*. He subjected eggs to oxygen, hydrogen, and to oxygen and carbon dioxide. The first one gave marked evidence of acceleration, as follows (table from Jenkinson):

Hours.	Oxygen.	Controls.
3	First furrow in some.	No furrow.
3½	All but one with first furrow.	Most with first furrow.
4	All but one four cells.	All with two cells.
5	All with four cells.	Most with two cells; a few with four.
47	Blastopore closed	White hemisphere visible.
73	Medullary fold.	Blastopore closed.

The experiments were carefully done so that matters of pressure and other disturbing factors were controlled, and the results may be attributed to the factor under consideration. The data from the experiments with hydrogen seem less conclusive as to the effects upon rate of cleavage, while no segmentations were obtained in the carbon dioxide experiments.

The effect of sodium hydrate upon *Arbacia* eggs was studied by Loeb. He found that the development and growth can be accelerated if the solution be made weakly alkaline, the concentration of sodium hydrate used being very small, perhaps .006 per cent. to .008 per cent. Acids have only an inhibiting effect. The chief cause for these effects must be that the oxidative processes in living substance are favored by the weak alkali, while acids decrease the oxidative processes and thereby inhibit syntheses. Loeb found that more than .2 cubic centimeter of 1/10 normal NaOH would not go into solution in 100 c.c. of sea water, for the amount of precipitate formed was only increased. Cleavage in the alkaline solution was slightly accelerated, but the amount of the increase was difficult to detect for any particular stage; as development progressed the effects became more clearly recognizable, and by the time the swimming blastula stage is reached the difference had become so pronounced that movement clearly began earlier in the eggs in the alkaline solution. In one case fertilization took place at 9:30 and at 3:15 the embryos in the alkaline sea water were swimming in lively manner, while the embryos in normal sea water remained motionless. In another case fertilization was at 11:00, 50 per cent. of the embryos in the alkaline sea water were swimming at 4:20, although all were motionless in the normal, and the first signs of movement in the latter were seen at 4:40. Loeb showed that the effects of the alkali were not simply upon ciliary movement, but also upon the development of the eggs themselves, for the next morning after such an experiment as the one just described the embryos in the alkaline solution would be in the *pluteus,* for example, and the normals in the round gastrula stage. And the alkali produced a difference not only in acceleration of the rate, but also an increase in the size of the plutei.

A similar action to that of the hydroxyl-ions was obtained by Mathews by the employment of pilocarpine hydrochlorate on *Asterias* larvæ. He found that the pilocarpine hastens the development and gives rise to abnormally large embryos, while atropine hindered the development and gave rise to dwarf embryos. Both of these drugs act directly upon the animal cells, and the nature of their action suggests that the atropine inhibits the oxidations taking place in those cells, while the pilocarpine increases those oxidations.

All of these agencies have the effect of accelerating the rate of cell division. In most cases the effect is only a slight one, but some of the stimulants are marked in their efficiency. However, even slight effects are significant if they are constant, and a careful analysis of the means by which acceleration even of slight amount may be produced will doubtless give additional light on the mechanism by which cell division is brought about.

Experiments with *Haminea* Eggs.

During the summer of 1921 the writer studied the effect of a number of accelerants upon the cleavage of the eggs of the opisthobranch *Haminea virescens* (Sowerby) at the laboratory of the Scripps Institution for Biological Research at La Jolla, California. The eggs of this animal are particularly favorable for experiments in which it is desired to test the effect of some special factor while leaving the egg in an environment that is normal in all respects except that investigated. The animals were brought into the laboratory and kept in a dish of running sea water supplied with a quantity of stones and sand from the tidal flat where they were first secured. Although they would at length become exhausted, no difficulty was experienced for some days in getting them to produce eggs.

The eggs are laid in a jelly mass which has the appearance of a short piece of narrow but very thick ribbon. It is of rather complicated structure. The eggs appear to be extruded in a string of tough gelatinous material which becomes surrounded by the matrix jelly forming the body of the ribbon. The string itself is laid in a zigzag fashion, so that the appearance is that of a double row of eggs. It is, however, accurately placed in the form of a flattened spiral so that the loops are not formed by simple back and forth folds as they at first appear, but are so arranged that the loops are compressed against each other. This produced the effect of a thick cross-striated ribbon. In one typical ribbon 242 loops were counted, in each of which the eggs averaged 90; this gave a total of 21,780 eggs for this ribbon. Probably 20,000 is an average number for a ribbon produced under typical conditions.

In each ribbon the eggs are uniformly all in the same stage of development, indeed in the same stage of mitotic division. It is a remarkable fact that 20,000 eggs should be deposited in as complicated a manner as these and all be in the same stage of division. But it is this fact in connection with the ribbon-like egg case that renders them desirable for experimental purposes. In conducting the experiments a ribbon would be cut into segments, one or more of which would form a control, while the others would be placed in the various solutions as desired and the results noted in comparison to the control.

The purpose of the first experiments upon these eggs was to verify and to extend Loeb's observations on *Arbacia* eggs that a small amount (.006 per cent.) of sodium hydrate would accelerate development. A considerable number of experiments planned to determine the effect of various concentrations of NaOH on the eggs were carried on. The general results of these were two. Some acceleration of cleavage resulted if the eggs were allowed to develop in sea water containing from .004 per cent. to .009 per cent. NaOH. Acceleration of cleavage does not always result in an earlier hatching of the larvæ, for it would seem that the advantage gained in the early stages sometimes takes expression in more vigorous larvæ rather than in more rapidly developing larvæ. In some experiments, indeed, hatching seems actually to be delayed by the treatment. A summary of the experiment numbered 156 is here given as an example of the sodium hydrate effect. Many other experiments were performed to verify these points, but they need not be given here.

Experiment No. 156. July 26, 1921. Began at 6:50 A.M. Eggs in 4-cell Stage.

6:50 A.M.	7:25 A.M.	8:45 A.M.	9:20 A.M.	12:00 A.M.	2:30 A.M.
156.1 Control (10 c.c. sea water)	4 cell	12	12–16	20	24–28 cell
156.2 0.2 c.c. 1/100 per cent. NaOH (in 10 c.c. sea water)	4–8	12	12–16	20	24–28 cell
156.3 0.4 c.c. 1/100 per cent. NaOH (in 10 c.c. sea water)		12	16	20–24	32–36
156.4 0.8 c.c. 1/100 per cent. NaOH (in 10 c.c. sea water)	4–8	12–16	16–20	20–24	32–36
156.5 1 c.c. 1/100 per cent. NaOH (in 10 c.c. sea water)	4–8	12–16	16	20	28

7/29, 10:00 A.M. 156.1 Larva large, moderately active.
156.2 Larva active, rotating.
156.3 Larva active, healthy.
156.4 Larva active, rotating.
156.5 Dead.

The effect of ammonium hydroxide is seen in experiment 159, for example. Here, too, the acceleration is evident. It is a little more difficult to obtain accurate results with ammonia since the concentration of the solution changes. I think, however, that the effects are not different in character from those of the sodium hydrate, although they are perhaps less evident in this case.

Experiment No. 159. July 31, 1921. Began at 7:10 Eggs Just Completed 3rd· Division.

7:10 A.M. Early 8 cell.	7:50 A.M.	9:20 A.M.	10:25 A.M.	11:50 A.M.
159.1 Control (10 c.c. sea water)	8 cell	16	20	28–32
159.3 0.6 c.c. 1/100 per cent. NH_4OH (in 10 c.c. sea water)	8	16	20–25	32
159.4 0.9 c.c. 1/100 per cent. NH_4OH (in 10 c.c. sea water)	8	12–16	20–24	32–36
159.5 1.2 c.c. 1/100 per cent. NH_4OH (in 10 c.c. sea water)	8	12–16	20	32

Potassium hydroxide is also similar in its effect, but the eggs respond with the more rapid division rate only to a slightly stronger concentration than in the case of NaOH. Experiment 161 furnishes an example of the KOH effects.

Experiment No. 161, August 2, 1921. Began at 7:00 A.M. Eggs in 4-cell Stage.

7:00 A.M. 4 Cell.	7:45 A.M.	8:45 A.M.	9:40 A.M.	10:30 A.M.	12:15 A.M.
161.1 Control (9 c.c. sea water)	8	12	16	20	24–28
161.2 0.3 c.c. 1/100 per cent. KOH (in 10 c.c. sea water)..	8	12	16	20	24–28
161.3 0.6 c.c. 1/100 per cent. KOH (in 10 c.c. sea water)..	8	12	16–20	24	28–32
161.4 0.9 c.c. 1/100 per cent. KOH (in 10 c.c. sea water)..	8	12	20	24	32
161.5 1.7 c.c. 1/100 per cent. KOH (in 10 c.c. sea water)..	8	12	20	24	24–28

8/8 7:00 A. M.	8/9 6:45 A. M.
161.1 ¾ hatched.	all hatched.
161.2 2/3 hatched.	8/10 hatched.
161.3 9/10 hatched.	all hatched.
161.4 A few hatched.	never hatched.
161.5 All hatched.	

It was desired by the writer to try the effect of various other hydroxides, especially those belonging to the first periodic group; but in the time at his disposal for the experiments he was able to obtain only two others, namely, barium hydroxide, $Ba(OH)_2$, and chromium hydroxide, $Cr_2(OH)_3$. Eggs were placed in solutions containing, respectively, .005 per cent., .006 per cent., .009 per cent., and .01 per cent. of barium hydroxide. In one experiment it seemed that some slight acceleration of the cleavage rate was probably affected by the reagent, but it was not possible afterwards to repeat that result and the writer is therefore disposed to believe that some other factor must have been responsible for the response observed. With chromium hydroxide also no acceleration could be seen in the cleavage rate.

Several experiments upon the effect of thyroid extract upon the cleavage rate were tried. A preparation of Parke Davis's desiccated thyroid was tried, with somewhat varying results. The experiments justify the statement that thyroid contains some agent which is able to increase at least slightly the rate of cleavage in these eggs, but the nature of the desiccated gland and the conditions of the experiments do not lend themselves to an exact statement of the effect itself or of the strength of material necessary to produce it. It is to be regretted that there was no further opportunity to study this matter in detail upon *Haminea* eggs.

Finally, influenced by Mathews's finding that the development of *Asterias* larvæ is hastened by pilocarpine hydrochlorate, the writer investigated the effect of this reagent upon *Haminea.* Mathews did not record any direct observations on the rate of cleavage, although an acceleration is to be inferred from his general results. The effect of pilocarpine on *Haminea* eggs was tried on concentrations of .1 c.c., .2 c.c., .3 c.c., .4 c.c., and .5 c.c. of ½ per cent. solution in 10 c.c. sea water. It was found that for these concentrations the effect, although slight, is an acceleration in any one cleavage in proportion to the concentration. The effect is less marked than in the case of the hydroxides of the first periodic group. Experiments 172.1 and 172.4 will serve as examples of the effects of pilocarpine hydrochlorate treatment. Pilocarpine nitrate was also tried, but the acceleration was not obtained by its use. It is probable that it is only the hydrochlorate which possesses the property of accelerating division rate.

EXPERIMENT NO. 172. AUGUST 16, 1921. BEGAN AT 6:35 A.M. EGGS JUST COMPLETED 2ND DIVISION.

6:35 8 Cell.	7:05 A.M.	7:55 A.M.	9:50 A.M.	8/18.
172.1 Control (10 c.c. sea water).	8	12–16	24	Rotating slowly.
172.4 0.3 c.c. $\frac{1}{2}$ per cent. pilocarpine in (10 c.c. sea water)...	8–12	16	24–28	Rotating, some rapidly.

DISCUSSION.

These experiments have raised a number of questions that the writer was unable to investigate because of the shortness of time at his disposal. In continuing the work it is necessary to use other material than *Haminea* eggs, a condition which is to be regretted, since these eggs are so favorable for this study. Work is nevertheless going on in this laboratory at the present time in an attempt to extend and develop certain of these conclusions and to clear up some of the questions.

If the list of hydroxides which cause acceleration be inspected, it is noted that they are the hydroxides of elements which belong to the first group of the periodic series (with the exception of ammonium, which, however, behaves chemically as do the members of that group). It is to be noted also that the hydroxides of barium of the second group and of chromium of the sixth group fail to induce the acceleration. One may suppose that it is only hydroxides of the first group that are effective in causing acceleration. This tentative conclusion is the object of certain experiments now being carried on, which at the date of the writing seem to bear it out. It is true that only hydroxides of the first group have any high degree of solubility, but this is discounted by the fact that the amount of any hydroxide which will go into solution in the already slightly alkaline sea water without causing precipitation is very small.

These results, I think, throw light upon the nature of the mitotic process and upon the mechanism by which it is accomplished. Practically all of the means now at our disposal for accelerating the rate of mitotic divisions are in line with increased oxidations and increased metabolism of the cells. Furthermore, a number of these accelerating agents are already known to affect favorably enzymatic activity. For example, the radium and x-ray studies previously referred to indicate that the accelerating and retarding effects of radiation are produced upon the enzymes of the cells. In this connection it is to be recalled that Mathews and others have suggested that the mitotic processes are correlated with the setting free and the activation of intracellular enzymes. The changes in the rate of enzyme action are able to bring about changes in the rate of division; and since the radiations affect the enzymatic activity, it is to be supposed that the changes which they induce in the rate of division are to be accounted for on the basis of this chemical mechanism. These considerations lead the writer to suggest that the agents which have been shown (either in this paper or in the list on a preceding page) to be capable of accelerating division do so because they possess the property of activating, either directly or indirectly, the inactive enzymes of the cells, or by increasing the activity of those which have already been activated. This line of approach offers a most profitable opportunity, it would seem, for the analysis of the forces and mechanism of mitosis.

SUMMARY.

The list of agencies which have been previously found to accelerate the rate of cell division includes heat, x-rays, radium, thyroid secretion, suprarenal extract, alcohol, dibasic potassium phosphate, potassium sulphate, potassium bromide, oxygen, sodium hydroxide, and pilocarpine hydrochlorate.

The results of the experiments on the eggs of *Haminea virescens* are in harmony with those of previous investigators. These experiments show that the eggs may be induced to undergo cleavage at an accelerated rate (although the amount of the acceleration need not be great) by the following reagents: .004 per cent. to .009 per cent. $NaOH$, .006 per cent. to .009 per cent. NH_4OH, .006 per cent. to .017 per cent. KOH, thyroid extract and pilocarpine hydrochlorate in weak concentrations. The accelerating effect of the pilocarpine is less than that of the hydroxides mentioned.

Barium hydroxide, chromium hydroxide, and pilocarpine nitrate cause no acceleration.

The experiments suggest that the hydroxides which are effective in causing acceleration of cleavage are those of elements which belong to the first group of the periodic series, and that those of other groups are ineffective in these respects.

Acceleration of cleavage is not always followed by continuously quickened development, for in some cases the experimental eggs do not hatch ahead of the control. The advantage gained in the segmentation stages may later manifest itself in more vigorous larvæ rather than in more rapidly developing ones.

The conclusion is also suggested that the agencies which are capable of accelerating division bring about this result through their property of influencing the enzymes of the cells, the setting free and the activation of which are correlated with the mitotic processes.

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BIOLOGICAL BULLETIN

ASSOCIATION OF HOMOLOGOUS CHROMOSOMES IN TETRAPLOID CELLS OF DIPTERA.

C. W. METZ,

Department of Genetics, Carnegie Institution of Washington.

A characteristic feature of chromosome behavior in the Diptera is the paired association of homologous chromosomes[1] in the diploid cells, somatic as well as germinal. This association is particularly striking in the early prophase of each cell generation, when the two homologous chromosomes are closely approximated—so closely, indeed, as to resemble a single element in many cases.[2] Like the association that occurs during synapsis in the germ cells of most organisms, this is apparently the result of an attraction between similar or identical elements in the conjugating chromosomes.

In considering the nature of the attractive force involved here, one of the first questions to arise is whether an equilibrium is established when two homologous chromosomes associate (analogous to a magnetic attraction) or whether the attraction extends to an indefinite number of members, so long as they are homologous. The observations on sporogenesis in certain triploid and tetraploid plants by El. and Em. Marchal ('11), Digby ('12), Osawa ('20), and Belling ('21) indicate that in the metaphase following synapsis homologous chromosomes are frequently grouped together, although not uniformly so. This indicates that there is, sometimes at least, an attraction between homologous chromosomes in numbers greater than two; but it tells little about the detailed nature of the association.

The observations of Holt ('17), of Metz ('16), and of Bridges ('22) on multiple chromosome groups of somatic cells in certain flies[1] have shown that the chromosomes regularly lie in clusters during metaphase, and that each cluster consists of the three, four, or more homologous members.[2] In prophase (Holt, '17; Metz, '16, Figs. 109, 110) the association of homologues is closer than in metaphase. Here again, however, there is some doubt about the exact nature of the association. Holt's observations on *Culex* seem to lead to two different views on this question, without indicating which is correct. According to one view sister chromosomes associate more closely than homologues that are not sisters. Evidence cited on p. 612, for instance, ". . . suggests that a parasynaptic union of sister chromosomes takes place in the telophase. . . ." This is supported also by her Figs. 13, 14, and 15, in which each original pair of chromosomes seems to be represented by two associated bodies, each of which is presumably made up of four or more sister chromosomes. If this is the case, the association between sister elements is closer than that between homologous, non-sister elements. But the statement is also made (p. 613) that "it is believed" that in multiple complexes the chromosomes conjugate "in three groups of homologous individuals" (three being the haploid number in *Culex*). The latter statement implies that there is no distinction between homologous and sister chromosomes.

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[1] First described by Stevens in 1907 and 1908.

[2] See Metz, '16, for a detailed account.

In the observations of the writer (l.c.) on *Fucellia* no attempt was made to analyze the details of the prophase association or to discriminate between sister and non-sister homologues.

The present paper is concerned with additional evidence on this point. The data are taken from some exceptionally clear prophase figures obtained in tetraploid ovarian cells of *Sarcophaga*.[3]

The normal chromosome group of *Sarcophaga* consists of five pairs of long chromosomes and one pair of short (sex) chromosomes. These are represented in Fig. 1, taken from a spermatogonium. In the early prophase, when the association of homologues is intimate, the six pairs resemble six single chromosomes, as shown in Fig. 2 (ovarian cell). The conditions found in tetraploid cells are represented in Figs. 3 to 6. These cells were found scattered about in the somatic tissue of the same ovary from which Fig. 2 is taken. The tetraploid cells are so large that the nucleus and some of the chromosomes are usually cut in sectioning; but a few nuclei have been found entire, or nearly so. One of these, a metaphase, is represented in Fig. 3. This figure brings out the loose association of chromosomes, characteristic of the

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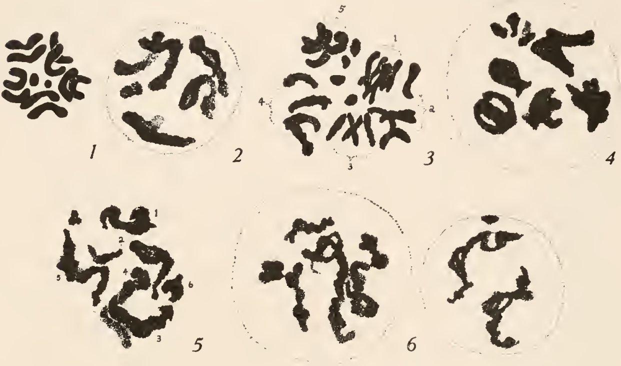
[1] Holt's observations were on *Culex;* those of Metz were on *Sarcophaga* and *Fucellia;* those of Bridges were on *Drosophila.*

[2] That the associating chromosomes are homologous cannot be doubted in the light of present cytological and genetical knowledge.

[3] The species has not been identified in this case, but all of my *Sarcophaga* material, including several identified species, shows essentially the same (normal) chromosome group throughout.



Explanation of Figures.*

All figures were drawn to the same scale, with the aid of a camera lucida. Numbers 1 and 2 are from diploid cells; numbers 3 to 6 are from tetraploid cells; numbers 2 to 6 are from the same ovary.

Fig. 1. Typical metaphase (from a spermatogonium) showing the chromosomes loosely associated in pairs. The smallest pair is the sex chromosome pair (XY).

Fig. 2. Typical prophase (ovarian cell) showing the close association of homologous chromosomes.

Fig. 3. Tetraploid chromosome group in metaphase showing loose association in fours instead of twos. The four smallest elements are the sex chromosomes (XXXX).

Fig. 4. Late prophase of a tetraploid nucleus showing close association in fours.

Figs. 5 and 6. Early prophases of tetraploid cells showing intimate association of homologous chromosomes.

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* I am indebted to Miss Ruth Lincks for making the drawings for the figures.

metaphase. Fig. 4 shows the closer association of late prophase. The intimate association of the early prophase is represented in Figs. 5 and 6. In the former each of the long bodies (numbered 1, 2, 3, 4, 5) is composed of four thread-like chromosomes, closely approximated side by side. This nucleus is almost entire, but the body numbered 2 appears to be cut at the point where the number is placed. Number 6 is presumably the set of small sex chromosomes. Fig. 6 represents a slightly earlier prophase in which the threads are somewhat more attenuated. The nucleus here is cut and lies in two sections, so that only three or four of the long, tetravalent bodies are complete. These show the same intimate association of homologous threads, however, as do the similar ones in Fig. 5. In both of these figures and in other prophases of a corresponding stage the association of homologues is so close that the individual threads can only be distinguished here and there.

As the figures indicate, the four threads of each body in the early prophase appear to be associated in equal degree. I can find no indication of a closer association of sister elements than of non-sister elements within the tetrad.

It appears, then, that in these cases the force which brings homologous chromosomes together is exerted equally between the respective members when four are present.[1] This agrees with the observations of Holt on *Culex* if we accept the latter of the two views suggested above—that the chromosomes conjugate in three groups of homologous individuals, without any distinction between sister and non-sister homologues.

Possibly this equivalence of association applies only to the gross relationships and does not represent the actual interrelations beyond the limits of visibility. That is a question, however, which can hardly be answered by a study of somatic cells. In the case of synapsis in the germ cells it may be possible to answer it by means of genetical studies on "crossing over" in tetraploid or other multiple chromosome races.

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[1] The question might be raised here as to whether these chromosomes actually come together, or whether they owe their association to successive divisions of the orginal prophase chromosomes *in situ*. The latter view involves the assumption that such cells do not multiply, and that each case represents an independent origin from a diploid cell. The evidence is against such an assumption, for all stages of division (prophase, metaphase, anaphase, etc.) may be found in these cells, and in some pieces of tissue nearly all of the cells are of the same multiple chromosome type (*e.g.*, in *Fucellia*).

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THE GEOTROPISM OF THE SEA-URCHIN *CENTRECHINUS.*

G. H. PARKER,

Zoölogical Laboratory, Harvard University.

The long-spined sea-urchin, *Centrechinus antillarum* (Phil.), is abundant on the flats near the Miami Aquarium in Biscayne Bay, Florida. It is a common object of interest in the aquarium tanks, where it is found climbing up the stone-covered walls or perched on the top of the submerged rock-work. When transferred to a large glass jar filled with quiet or running sea water, it immediately starts to climb up the sides of the vessel, and each time it is loosened and dropped to the bottom it renews this activity. This response occurs in the dark as well as in the light, and in vessels the water of which is cut off from the air by a glass plate as well as in those whose contents are exposed to the air. In other words, *Centrechinus* climbs upward, not because of light or of access to oxygen, but in response to gravity. It is a strongly geonegative animal.

Centrechinus belongs to the group of regular sea-urchins, but, like most representatives of this group, its radial symmetry is disturbed by the single madreporite which occupies the aboral end of one of its five interradial zones. Thus a structural axis of orientation is established. As *Centrechinus* is remarkably active in its geotropic responses, it is a favorable form with which to test orientation in locomotion. Does it move as bilateral animals almost always do, with one end of a definite axis constantly forward, as, for instance, the one marked by the madreporite, or may any of its numerous axes serve as a line of progression? This general question has already excited the attention of a number of students of the echinoderms.

Jennings (1907, p. 155) states that the California starfish, *Asterias forreri,* "may move with any one of its rays in the lead, or with any interradius in advance, or indeed in any intermediate direction, so that its possibilities as to variations of direction of locomotion are really unlimited." Cole (1913) in his study of the New England starfish, *Asterias forbesi,* demonstrated in this species the same unlimited possibility of direction of locomotion that Jennings had done for *A. forreri,* but Cole further showed by means of statistical methods that *A. forbesi* moved more frequently with that part forward which was in close proximity to the madreporite than with any other part. He, therefore, established what has been called a physiological anterior for this species, an anterior that corresponds almost exactly with that assumed on different grounds by Lovén for echinoderms in general. Cole's observations form, as a matter of fact, a rather remarkable confirmation of Lovén's deductions.

Agersborg (1918, p. 233) in his study of the habits of the twenty-rayed starfish of the Pacific coast, *Pycnopodia helianthoides,* calls attention to its bilateral tendencies and states that it does not readily move backward or sidewise, but uses the side established as fore end always as anterior end. He, however, nowhere makes really clear how its physiological bilaterality is related to its structural bilaterality. If they agree, as his account seems to imply, then the physiological anterior of *Pycnopodia* must be different from that of *Asterias,* judging from the account of the structure of *Pycnopodia* given by Ritter and Crocker (1900). In *Asterias,* according to Cole, the physiological anterior centers on the arm to the left of the madreporite as viewed aborally (III. in Lovén's system). In *Pycnopodia* it appears to center on the second arm to the right from the madreporite as viewed aborally. Thus the axis of locomotion in *Asterias* is two fifths of a circumference from that in *Pycnopodia.* But this conclusion is based on statements of structure from Ritter and Crocker and of habits from Agersborg, both of which may be open to fundamental revision. So far as *Pycnopodia* is concerned, the one thing that in reality seems certain is that it has a physiological anterior, but how this is related to its structure remains to be ascertained.[1]

The Bermudian starfish, *Coscinasterias tenuispina,* has been studied in its methods of reproduction and of locomotion by Crozier (1920*a*). This species propagates by division and consequently a given individual may have more than one madreporite as a preparatory step to division and may have arms of different length as the result of regeneration after division. Crozier studied the locomotion of *Coscinasterias* in relation to the length of the arms and the position of the madreporites and found that both factors had an influence in determining the axis of motion. Although the animal could move in any direction, there was an obvious tendency to move more generally in the direction of the long arms and on the axis determined by the position of the madreporite, and of these two factors the position of the madreporite on the whole predominated. Thus in all the starfishes that have been investigated in this respect a physiological anterior has been discovered, and this anterior seems to find a structural indication in the position of the madreporite whereby an arm next this organ commonly becomes a director.

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[1] This subject is not greatly clarified in a second paper recently published by Agersborg (BIOL. BULL., vol. 42, p. 202).

Among the crinoids Clark (1915) has shown that *Comatula purpurea* moves with its long arms anterior, but whether the axis thus established agrees with that in the starfishes can not be ascertained because of the absence of the madreporite from crinoids. *Comatula* must, however, be admitted to possess a physiological anterior.

Few observations have been made on the direction of locomotion in the sea-urchins. Holmes (1912), in his study of the phototropism of *Arbacia*, describes the locomotion of this sea-urchin as though it were free to move in any direction, yet he makes no specific statement on this point. Crozier (1920*b*), who studied the bionomics of the sand-dollar, *Mellita,* found that this animal had a very definite axis of locomotion which corresponded with its structural axis of bilaterality. In *Mellita,* as in other sand-dollars, the mouth is at the center of the underside of the disc, but the anus is on the edge of the disc in an interradial position. A line drawn through the anus and the center of the disc in *Mellita* marks the axis of locomotion and the forward end of this axis in creeping and in digging is the end farthest from the anus. The madreporite in the sand-dollar is central in position and hence can not be used in homologizing rays as in other sea-urchins, but by comparing the conditions in *Mellita* with those in the spatangoids, in which the symmetry is also bilateral but the madreporite is excentric, it can be shown that the ray in the sand-dollar that is opposite the anus is the homologue of the ray in *Asterias* that is to the left of the madreporite and that serves as the director in locomotion. Hence the axis of symmetry and direction of locomotion in *Mellita,* as determined by Crozier, are in agreement with the conditions in *Asterias* as determined by Cole. This is a rather striking correspondence in both anatomical and physiological details.

Although the radial symmetry of the echinoderms expresses itself in the ability of these animals to move in any direction in the horizontal plane, yet in the few instances studied there seems to be also a marked tendency toward a physiological anterior which takes its origin from the position of the madreporite and is in agreement with the structural bilaterality of the spatangoids and the clypeastroids.

To test these relations in the regular sea-urchin *Centrechinus* a bit of white thread was tied to a spine on the ray to the left of the madreporite (III. in the Lovén system) and, with this ray thus identified, the sea-urchin was allowed to climb the sides of a glass jar ten times and the position of the axis of locomotion in relation to the given ray was recorded for each ascent. Tests of this kind were made on four animals. These animals, after an ascent had begun, showed little of the circling movement observed by Crozier (1920*b*) in *Mellita,* but maintained a fairly constant relation between the axis of locomotion and their structural axis over a vertical course of some 40 centimeters. It was not always easy to observe the exact relation of the structural axis of the animal, as indicated by the marked spine, to the direction of locomotion, but this relation is accurately enough indicated in terms of rays, though the animals may have crept at times more nearly interradially than radially. Records were kept by noting which ray was nearest the physiological anterior during the test. The results are shown in Table I.

Two conclusions may be drawn from Table I. First, there is nothing about the records in this table that suggests that *Centrechinus* has a physiological anterior. The ray III., that might be suspected in this respect, is in no sense a director, in fact it is rather the reverse. It is, however, perhaps open to doubt whether there is a sufficient number of observations in the table to warrant any sound conclusion whatever on the question of a physiological anterior. But so far as these records go, there seems to be no suggestion of this state in *Centrechinus.*

Table I.

Number of Times in Ten Trials on Each of Four Sea-urchins (*Centrechinus*) that a Given Ray was Foremost in Geonegative Locomotion.

The rays are numbered according to the Lovén system, the ray opposite the madreporite being V, the one to the right of this ray being I. and the others following in sequence around to V.

No. of Animal.	No. of Ray (Lovén's System).				
	I.	II.	III.	IV.	V.
1	2	2	1	3	2
2	2	3	2	2	1
3	2	1	3	3	1
4	3	2	1	1	3
Totals	9	8	7	9	7

The second conclusion to be drawn from Table I. is abundantly supported by the observations. This conclusion is to the effect that *Centrechinus* is able to carry out geonegative responses on any axis in its body. This conclusion is unquestionable and shows that the geotropism of *Centrechinus*, unlike that of a bilateral animal, is performed without initial orientation and, as a type of sea-urchin locomotion, it is not necessarily associated with any particular ray. In this respect the geotropism of *Centrechinus* is much more like that of a plant than like that of most animals in which the axis of the animal is first moved so that the creature heads either toward the center of the earth or away from it, after which locomotion in the appropriate direction takes place. In *Centrechinus,* as already intimated, no initial orientation occurs, but the sea-urchin with any axis forward creeps upward. This condition emphasizes what Loeb long ago pointed out, the essential similarity between the tropisms of plants and of animals.

When an attempt is made to analyze the geonegative responses of *Centrechinus,* it is found to be no simple problem. These responses involve stereotropism as well as geotropism and the latter in its more generalized type such as occurs in plants. If a *Cen-*

trechinus is dropped so as to rest with its side or its aboral face on the horizontal floor of an aquarium, an activity of spines and ambulacral feet ensues which eventually rights the animal in that its oral side is brought next the aquarium floor. Not till righting has been accomplished does locomotion in the proper sense begin. I never have seen a *Centrechinus* progress on its back or its side, but only with its oral surface next the substrate. It has often been assumed that in echinoderms the righting reaction is a response to gravity, but this is doubtful, in *Centrechinus* at least, as the following experiment shows. If a strong thread is tied round the equator of a *Centrechinus* and the animal is suspended in an aquarium so that its side touches the vertical glass wall, the spines and feet will begin the same kind of movements that they did when the animal rested on its side at the bottom, and in a short time the sea-urchin will have its oral surface applied to the vertical face of the aquarium as it formerly did to the floor. Since in this test the axis of the sea-urchin is at right angles to the direction of gravity instead of being parallel to it as in the ordinary righting reaction, it is clear that righting is not a response to gravity, but to a solid surface against which the creature comes always to apply its oral side. Righting in *Centrechinus,* therefore, though apparently geotropic, is in reality not so, but stereotropic. On this point my results confirm those of Moore (1910), who has described a similar condition in the starfish. In discussing the geotropism of *Centrechinus,* therefore, the righting movements are not to be considered, for they belong to a different category of reactions; they are reactions to solid surfaces. In this respect *Centrechinus* agrees with *Planaria,* whose righting reactions are apparently also stereotropic (Pearl, 1902), though this worm likewise shows true geotropism (Olmsted, 1917).

After a *Centrechinus* has obtained a footing on a vertical surface, it does not wander indiscriminately over this surface, but it travels up the surface against gravity. It is this reaction that is indicative of true geotropism and that still requires to be considered.

Two general theories have been advanced to show how geotropic responses are accomplished: the so-called mechanical theory apparently first proposed by Aderhold (1888) and advocated by Verworn (1889) and the statocyst theory put forth by Lyon (1905) and espoused by a number of later workers, Kanda (1914) and others. According to the mechanical theory the weight of the body of the responding organism is so disposed in relation to the locomotor apparatus that the creature is kept mechanically headed either toward the center of the earth or away from it. In this way the action of gravity on the body as a whole determines the direction of locomotion. In such cases the organism would maintain the same orientation dead or alive. Few, if any, instances of this kind have ever been found, and though it must be admitted that every organism is always under the direct influence of gravity and varies in weight in different parts of its body, practically none have been found that exhibit conditions favorable to this view. *Centrechinus* with its symmetrical distribution of parts gives no suggestion of a preponderance of weight on one side or the other, and the fact that it may assume a new axis with almost every ascent on the aquarium wall is a condition extremely difficult for the mechanical theory to meet.

The statocyst theory assumes that within the body of the organism there are movable masses of higher specific gravity than their fluid surroundings, and that these masses under the influence of gravity press upon one side or the other of their containing chambers in accordance with the position of the organism in relation to the center of the earth. To these pressures the organism then responds in an appropriate way. Organs acting in this fashion and known as statocysts with their contained statoliths occur commonly among such animals as the worms, the mollusks, and the crustaceans.

This theory is also believed to apply to the simpler animals and to the plants in that geotropism is excited in these organisms by the pressure exerted by small particles contained within the vacuoles of their protoplasm. This view is supported by such observations as those of Zollikofer (1918) on the seedlings of certain compositæ. When these seedlings are placed in the dark for three or four days, the starch grains in their hypocotyls disappear, and with the disappearance of the starch the geotropic reactions of the seedlings cease, though the seedlings seem not to have suffered from the treatment, for they are still phototropic. Apparently the starch grains act as statoliths in the spaces in which they are lodged. In this way the small solid particles in the protoplasm of the simpler animals and plants are supposed to render these organisms geotropic.

As a refinement and extension of this view, Small (1920) has suggested that under gravity the disperse phase in the protoplasm of geotropic organisms moves to one side of their bodies, a process analogous to creaming, and thereby brings about a change in electrical potential which results in geotropic movements. As an objection to this view Blackman (1921) points out, among other things, that creaming is a slow process, and that geotropic response is often quick. Certainly in *Centrechinus* such a response can occur in less than a second, as can be shown by tilting into the vertical a horizontal plate of glass on which a sea-urchin is creeping. Hence creaming can scarcely explain the geotropism of the sea-urchin.

How this is to be explained is not a simple matter, for *Centrechinus,* so far as is known, is entirely devoid of statocysts, and even assuming that it possessed them, the fact that it progresses upward with any axis forward is a difficult feature to explain. Nevertheless its body is provided with a number of parts whose action may make clear how its geotropism is accomplished. Such parts are the spines and the ambulacral feet. They are heavier than the sea water in which they are immersed and in consequence of their weight tend to hang down from their supports. This is especially true of the spines, which are heavily impregnated with lime and provided with ball-and-socket bases. In fact, these organs are beautifully arranged to respond to the pull of gravity and, assuming that their bases are provided with a nervous mechanism sufficiently differentiated for the purpose, they might perfectly well serve as organs for the initiation of the response to gravity. In that case the stimulus would be the deforming pressure exerted at the base of the spine by its movement under the pull of gravity. It is a deforming pressure of this kind that acts as a stimulus and not a general pressure such as fluids exert and as was believed to be effective in geotropism by Jensen (1893). A deforming pressure, if exerted locally at the base of the spine, might well excite in a differentiated system of basal receptors impulses to locomotion that would result in an appropriate geotropism. Such a form of response is in essence that implied in the statocyst theory in which the stimulus must be a deforming, not a general, pressure. But whether in *Centrechinus* it is the spines or some other similarly constructed organ that initiates the geotropic response remains to be ascertained.

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INITIATION OF DEVELOPMENT IN THE EGG OF *ARBACIA*.

I. Effect of Hypertonic Sea-water in Producing Membrane Separation, Cleavage, and Top-swimming Plutei.

E. E. JUST,[1]

Rosenwald Fellow in Biology, National Research Council.

It is well known from Morgan's work that unfertilized eggs of *Arbacia* may be induced to develop through exposure to hypertonic sea-water. Morgan, however, did not investigate the action of hypertonic sea-water on the unfertilized egg much beyond its effect in producing cleavage. Loeb extended these results of Morgan: he was able by the use of hypertonic sea-water to produce plutei from the unfertilized eggs of *Arbacia*. Two outstanding features of Loeb's work strike the reader: first, he was not able with the use of hypertonic sea-water to call forth "membrane formation"; nor was he able to obtain plutei of great viability, since these failed to swim at the surface as do plutei from normally fertilized eggs.

With his now classic method of exposing urchins' eggs to butyric acid in sea-water before or after exposure to hypertonic sea-water, Loeb was able to correct both these defects. On the basis of these findings Loeb founded his famous lysin theory of fertilization. He reasoned that butyric acid, as all hæmolytic agents, brings about a "superficial cytolysis" of the egg and thus the formation of the "fertilization membrane." This "superficial cytolysis," however, tends to be lethal and hence the egg must have a corrective treatment to offset the initiation of death changes. The hypertonic sea-water acts as this corrective factor. According to Loeb, it is of no moment whether he uses the corrective agent first and follows with the cytolytic agent or *vice versa*. In other words, the "uncorrected" egg may be first corrected, then superficially cytolyzed; or the egg may be first superficially cytolyzed and saved from death by the corrective factor. In any event, it is clear not only from Loeb's work, but that of others, that this double treatment of the eggs of sea-urchins produces top-swimming larvæ.

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[1] Zoölogical Laboratory, Howard University.

Loeb's work with agents of superficial cytolysis and the corrective factor led him to solve the fertilization problem in this wise: The sperm carries a lysin which initiates a superficial cytolysis of the egg; thus the first effect of the sperm is comparable to the action of butyric acid. But the sperm, reasons Loeb, also carries a corrective factor which checks the action of the lysin that otherwise would kill the egg. This reasoning is aided by the fact that in many ova the internal changes of fertilization leading to cell division are preceded by demonstrable cortical changes.

I have attempted to point out that this theory of Loeb fails to explain fertilization, and this for several reasons. Waiving not only the fact that Loeb has produced cell division and swimming plutei from uninseminated urchin eggs with the use of hypertonic sea-water before or after the treatment with butyric acid, whereas in the fertilization of these eggs the cortical changes always precede the internal—cell division—phenomena, but waiving also the fact that hypertonic sea-water alone will give cleavage and plutei, we must discard the superficial cytolysis-corrective factor theory of fertilization for two reasons: First, this theory emphasizes too much purely hypothetical substances in the sperm for which we have not a single bit of evidence; and, secondly, it wholly ignores the fact that the egg is a highly irritable system, thus in no wise different from other living substance; that there are naturally parthenogenetic eggs would indicate this. Moreover, the high degree of susceptibility to shaking of such eggs as those of *Asterias, Amphitrite, Nereis,* and the effect of sea-water in starting up maturation in eggs of *Podarke, Chætopterus,* etc., show how labile are some uninseminated marine ova. This work on the experimental production of cell division and larvæ is of importance in showing that ova are independent, activable systems; they are inherently irritable—not a difficult physiological conception. But as a means of elucidating the problem of fertilization, this work on "artificial parthenogenesis," so called, has failed; it has actually obscured the fertilization problem.

For these two reasons, then, the superficial cytolysis-corrective factor theory of experimental parthenogenesis has no logical status as an explanation of fertilization. Fertilization can be explained only by observation and experiment on ova and sperm during fertilization. It can not be explained by mere analogy of the processes in experimental parthenogenesis.

But the superficial cytolysis-corrective factor theory as an explanation of experimental parthenogenesis itself is open to grave suspicion. First, the corrective factor may operate alone and give results. In the second place, the corrective factor, says Loeb, may act first when, according to the theory, there is nothing to correct, and the cytolytic agent may follow presumably to vitiate the action of the corrective factor. Again, as I have previously pointed out, the theory is largely built on the assumption that the proper exposure to butyric acid for inducing membrane formation is cytolytic because an over-exposure is lethal. This does not follow. One might just as well argue that since stimulation of the cardiac components of the vagus causes cessation of the heart beat the normal function of these fibers is to kill the animal.

Nevertheless some may hold, despite these criticisms, that the superficial cytolysis-corrective factor hypothesis is still a valid explanation of experimental parthenogenesis; that while it is true that most marine ova need but a single agent to induce development, eggs of sea-urchins need two. If, now, we can show for the egg of *Arbacia* that a *single* agent acting alone can induce both membrane formation and cleavage, then again is the famous theory put to question. And if, more than this, we can show that this single agent is the corrective factor—*anti-cytolytic,* if you please—then the superficial cytolysis-corrective factor theory must be rejected, for the egg of *Arbacia* at least, as an explanation not only of fertilization, but also of experimental parthenogenesis as well.

The present communication aims to present data, accumulated during the season of 1921 at the Marine Biological Laboratory, Woods Hole, Mass., to show that hypertonic sea-water alone acting on the uninseminated eggs of *Arbacia* will give membranes, cleavage, and viable surface-swimming plutei scarcely to be distinguished from those resulting from normally fertilized eggs.

I.

If the uninseminated eggs of *Arbacia* be exposed to sea-water made hypertonic by the addition of NaCl or KCl in the proportions 50 parts sea-water plus 8 parts 2½ *M* NaCl or KCl, on return to normal sea-water they are induced to cleave and develop plutei. The per cent. of eggs that develop depends upon the length of exposure which will vary somewhat with different lots of eggs. Too brief an exposure will call forth merely the monaster condition and few, if any, of the eggs cleave; too long an exposure will produce cytasters, the resulting cleavage being abnormal. These eggs do not form membranes.

If 15, 16, and 17 parts 2½ *M* NaCl or KCl plus 85, 84, and 83 parts sea-water, respectively, are employed, the results are similar to those obtained with the hypertonic sea-water mentioned above (in the proportion 8 parts 2½ *M* NaCl or KCl plus 50 parts sea-water). With hypertonic sea-water made up with 20, 22, and 24 parts 2½ *M* NaCl or KCl plus 80, 78, and 76 parts sea-water, respectively, however, the results are quite different. In these and stronger hypertonic solutions of sea-water *the eggs lift off membranes while in the solutions.* The time from the instant that one treats eggs with a solution to that at which the eggs form membranes will vary with the strength of the solution. Thus in full strength 2½ *M* NaCl or KCl eggs lift off membranes in 15 seconds. In the solution 24 parts 2½ *M* NaCl or KCl plus 76 parts sea-water the eggs lift off membranes in five to ten minutes. Solutions between these two strengths call forth membranes at rates proportional to the degree of hypertonicity. The rate at which eggs lift membranes while in the solutions depends thus upon the strength of the solution.

The solution 18 parts 2½ *M* NaCl or KCl plus 82 parts sea-water gives about 3 per cent. membranes. It is thus the minimum concentration for the production of membranes. Hypertonic sea-water below this concentration does not yield membranes.

On the whole, the optimum concentration is that which gives the highest per cent. of membranes and which likewise allows an exposure longer than that to produce membranes without any deleterious effect on the eggs as revealed by their subsequent fate on restoral to normal sea-water. Such an optimum lies around 22 parts 2½ M NaCl or KCl plus 78 parts sea-water. The solutions 20, 22, and 24 parts 2½ M NaCl or KCl plus 80, 78, and 76 parts sea-water, respectively, were the ones used most extensively. In general, portions of eggs from one female were exposed to each of these concentrations to cover any variation of the eggs with respect to their response to treatment with hypertonic sea-water, since these concentrations are around the optimum. The following table summarizes results of a part of the forty experiments on this point. It is scarcely necessary to say that in all this work extreme precautions were taken against accidental insemination. In none of the experiments did the control, uninseminated eggs in sea-water, show a single membrane.

TABLE I.

EFFECT OF HYPERTONIC SEA-WATER ON EGGS OF ARBACIA AS SHOWN BY THE PER CENT. OF EGGS THAT SEPARATE MEMBRANES WHILE IN THE HYPERTONIC SEA-WATER.

Date of Experiment.	Per Cent. of 2½ M NaCL[1] in Sea-water to which Eggs Exposed.	Per Cent. of Eggs that Separate Membranes.
June 26	14	0
“	16	–1
“	18	3
“	20	35
“	22	60
“	24	78
June 27	14	0
“	16	–1
“	18	3
“	20	20
“	22	46
“	24	56
June 30	20	70
“	22	93
“	24	84
July 1	20	94
“	22	78
“	24	63
July 25	20	48
“	22	69
“	24	94
July 30	20	83
“	22	87
“	24	80

[1] 2½ M KCL gives closely similar results.

This table shows, I think, that hypertonic sea-water alone will induce membrane separation. In the most successful experiments *every single mature egg showed a membrane.* Since, moreover, experiments were made throughout the season, the results can not be interpreted as mere incidental findings based on insufficient data.

These membranes induced by hypertonic sea-water separate more slowly than membranes lifted from the eggs following normal insemination. These membranes are, nevertheless, as clear and as distinct and possess as wide a perivitelline space as normally fertilized eggs. In the hypertonic sea-water the egg shrinks, its periphery retaining a smooth contour. One gains the impression that the perivitelline space arises in part as the result of this shrinkage. That this is not wholly correct seems to be indicated by those eggs that undergo an equal amount of shrinkage without forming membranes. Moreover, on return to sea-water the egg, though it increases in size, does not obliterate the perivitelline space.

If the intensity of the membrane separation process be too great, the membrane formed is eccentric; the perivitelline space is not of the same width in all zones of the egg. In such cases the egg, as seen in optical section, is flattened in that zone above which the membrane is at its greatest distance from the egg. On return of the egg to normal sea-water this eccentricity of the membrane persists. The cortex of that zone, in these cases, from which the membrane has separated least, is apt to be swollen. This seems to indicate that the reaction underlying membrane must be of a certain order to insure best results.

The membrane does not always arise in the manner described. In some cases the egg presents a crenated surface beneath the membrane. This crenation may quickly disappear, leaving the egg cortex below the membrane perfectly smooth. If the crenation persist, on return to normal sea-water the perivitelline space is very narrow; indeed, it may be absent, in which case the membrane is closely stuck to the swollen cortex.

Finally, in some cases the membrane may be extremely thin, though otherwise the egg and perivitelline space are about as found in the normally fertilized egg.

These observations on the effect of hypertonic sea-water in bringing about membrane separation, fortunately, do not stand alone. I find that Loeb almost twenty years ago made a similar observation on the egg of *Strongylocentrotus*. Using concentrated solutions (2½ and 1½ *n* NaCl and 2½ *n* and 2 *n* cane sugar), Loeb[1] found that the unfertilized eggs of *S. purpuratus* form membranes in the same way as in fertilization. The details of his description differ very little from those I have given above for the egg of *Arbacia*.

Moore, working with *Arbacia*, was able by the use of hypertonic sea-water alone (16 c.c. 2½ *M* NaCl plus 50 c.c. sea-water) to obtain "quite a considerable number of membranes." According to Moore, however, these membranes are not like the fertilization membranes produced in normal fertilization.

In those lots of eggs that show a high per cent. of immature eggs, some mature eggs may fail to show membrane separation in any concentration of hypertonic sea-water. Stale eggs often fail to respond to hypertonic solution with membrane separation. Blood inhibits membrane separation and enhances the cortical changes that give the thick swollen cortex. Eggs that fail to form membranes in the hypertonic sea-water are invariably from lots that yield a low per cent. of membranes following normal insemination. We may consider these points in detail.

On June 29, July 8, July 18, for example, uninseminated eggs were mixed with blood. In each experiment the eggs were divided into three lots—*A*, *B*, and *C*. *A* was untreated (control), *B* inseminated, and *C* exposed to hypertonic sea-water. Not a single egg in any of the lots *B* formed membranes. The lots (*C*) treated with hypertonic sea-water (20, 22, and 24 parts 2½ *M* NaCl plus 80, 78, and 76 parts sea-water, respectively) gave a low per cent. of very poor membranes; instead, in the majority the egg cortex became badly swollen. Nothing was more clearly brought out in the work than this sharp inhibition by blood both in fertilization and in experimental parthenogenesis.

Several experiments, for example, those of August 1, 2, and 3, were made on washed eggs. These established that eggs lose their capacity for artificial activation more quickly than their capacity for fertilization. In one case eggs washed but four times in an hour were highly fertilizable, as shown by the presence of 96 per cent. membranes and subsequent normal development.

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[1] *Pflüger's Archiv*, '04, 103.

Uninseminated eggs from this same lot exposed to hypertonic sea-water gave only 17 per cent. membranes. Eggs a day old that have been repeatedly washed never gave membranes with hypertonic sea-water, though they were capable of responding to insemination with complete membrane separation.

Immature eggs give no response to treatment with hypertonic sea-water, as experiments early in June revealed.

The best criterion, we may conclude, for the capacity of the eggs to respond to treatment with hypertonic sea-water is their response to insemination. Eggs from the same lots as those which, when inseminated, rapidly lift off fine membranes everywhere equidistant from the eggs with wide perivitelline spaces are the best for hypertonic sea-water treatment. Eggs in the presence of blood, stale eggs, and immature eggs, lift few or no fertilization membranes. Such eggs yield poor or no results with hypertonic sea-water.

I pass now to the consideration of the type of cleavage and plutei resulting from *Arbacia* eggs exposed to hypertonic sea-water (20, 22, and 24 parts 2½ *M* NaCl or KCl plus 80, 78, and 76 parts sea-water, respectively, and sea-water of greater hypertonicity). And I may say at the outset that the quality and per cent. of membranes separated in hypertonic sea-water are indices of cleavage and the production of plutei. The production of cleavage and of surface-swimming plutei are of the best quality and most numerous from those lots of eggs with best membranes, provided, always, that the exposure is optimum. Data on this point are summarized in Table II.

TABLE II.

PER CENT. OF CLEAVAGE AND OF PLUTEI FROM EGGS OF ARBACIA FOLLOWING EXPOSURE TO HYPERTONIC SEA-WATER IN WHICH THE EGGS SEPARATE MEMBRANES.

Date of Experiment.	Per Cent. of Membranes Formed in the Hypertonic Sea-water.	Per Cent. of Cleavage in Eggs on Return to Sea-water.	Per Cent. of Top Swimming Plutei (Estimated).
July 17	96	93	85
" 19	34	37	25
" 20	41	32	25
" 29	88	79	70
" 30	92	89	85
Aug. 1	84	77	65
" 2	0	7	0

The experiments here cited, a fraction of the total, show that the best cleavage and plutei, both as to quality and per cent., are invariably found in those eggs that produce the best membranes. At times the results are perfectly wonderful. Thus on August 9 eggs exposed the day before to KCl hypertonic sea-water gave gastrulæ (and later plutei) that were scarcely to be distinguished from those arising from normally fertilized eggs. The seven dishes were simply alive with surface-swimming forms. These eggs had lifted off very fine membranes. On the other hand, on August 6 the eggs treated with KCl hypertonic sea-water lifted off very poor membranes. They produced inferior cleavages and larvæ. The cleavage and larvæ resulting from exposure to sea-water of such concentration that membrane separation does not take place in no wise compare to those from eggs in which membrane separation takes place in hypertonic sea-water.

In my experience insemination of eggs on return to normal sea-water following an exposure to hypertonic sea-water that calls forth membrane separation is not possible. If the cortical reaction is complete and full membranes separate, insemination does not increase the per cent. of cleavage and of plutei. In eggs induced to form membranes by hypertonic sea-water the cortical reaction is therefore complete and irreversible.

The results here reported are in every way equal to those obtained with the butyric acid-hypertonic sea-water method. Indeed, in my experience the results with the use of the strong hypertonic solutions have proved superior to the butyric acid-hypertonic sea-water method. And certainly the use of hypertonic sea-water alone is far more simple. With butyric acid one must get just the right exposure for membrane separation. In any lot of eggs, a mixed population, all eggs do not have precisely the same optimum point of exposure to butyric acid for perfect membranes. Moreover, even with the very highest per cent. of membranes following butyric-acid treatment, the worker must again at intervals give the eggs exposure to hypertonic sea-water of various lengths. Three optima must the worker, therefore, obtain for best results: optimum exposure to butyric acid, optimum length of time in sea-water following butyric-acid treatment before exposure to hypertonic sea-water, and optimum exposure to hypertonic sea-water. With the hypertonic solutions used in the experiments here presented, the case is quite otherwise: one simply notes the time in hypertonic sea-water to membrane separation and allows roughly twice this length of time before removal to normal sea-water.

But the main point in these experiments, it seems to me, is not the inferiority or the superiority of this method of a single exposure to hypertonic to the butyric acid-hypertonic sea-water method. If the experiments here reported simply revealed that the single hypertonic sea-water treatment only calls forth membrane lifting, they would be, it seems to me, worthy of report. And for this reason: If hypertonic sea-water be capable of inducing membrane separation, then we must discard the superficial cytolysis-corrective factor hypothesis for experimental parthenogenesis, as we have already discarded this hypothesis as explaining fertilization. I propose, therefore, to discuss these results, since they involve to a far-reaching degree current conceptions of the mechanism of experimental parthenogenesis.

II.

The evidence submitted above shows (1) that sea-water, if made sufficiently hypertonic, is alone capable of inducing membrane separation in the eggs of *Arbacia;* (2) that such eggs give good cleavage and practically normal gastrulæ and plutei; and (3) that the highest per cent. and normality of cleavage and of plutei result when the membrane separation most closely simulates the separation of the vitelline membrane as a cortical response to insemination. If this be true, several important considerations follow with regard to the nature of the processes underlying membrane separation and to the interpretation of these processes in the physiology of the developing egg cell. These considerations follow:

1. In the first place, membrane separation certainly can not be due to any mere surface tension change. According to Traube,[1] substances are effective in calling forth membrane separation the more they lower surface tension. From this it follows that hypotonic sea-water should be capable of inducing membrane separa-

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[1] "Ueber Parthenogenese," J. Traube, *Biochem. Zeitschr.*, Bd. 16, 1909, pages 182–186. Cf. also, McClendon, *Am. Jour. Phys.*, 10, 27, 240.

tion. This is true, as Schücking found. Toluene, etc., should likewise be effective, and they are (cf. Herbst). But surely one could scarcely insist upon this same explanation for the effect of the hypertonic sea-water employed in the experiments here reported.

Moreover, in the eggs both of *Arbacia* and of *Echinarachnius* any competent observer can see that membrane separation following insemination is no mere surface tension effect, but an active progressive dissolution of cortical material. In the egg of *Echinarachnius* one can actually observe the cortex going into solution; in the egg of *Arbacia* pigment escapes at this time. If, therefore, we experiment with agents that induce membrane separation, in order to solve the problem of the cortical changes in normal fertilization; despite the fact that such agents do lower the surface tension of the sea-water, we are not justified in the light of the observed phenomena in normal fertilization to postulate any theory at variance with these observed phenomena. Such postulates must cease to have any scientific value.

To be sure, it may well be that the membranes induced by these agents are not at all comparable to those induced by sperm. Nor, indeed, does it follow that membranes induced by hypertonic sea-water are like those induced by sperm. The main point, however, is something more than this. Hypertonic sea-water, which certainly is not of lower surface tension than normal sea-water, does call forth membranes while the eggs are in the solution. If we must adhere to the surface tension hypothesis, then we must conclude that the effect of hypertonic sea-water is an exception—as is the effect of the sperm in calling forth membrane separation by a cortical breakdown which follows in a wave beginning at the entrance point of the sperm.

2. Again, the experiments here reported are at variance with the notion that the separation of the membrane is due to a superficial cytolysis.

As I understand it, the term cytolysis connotes a cellular disintegration. One certainly can not use the term in its strict etymological sense particularly since that misnomer "superficial cytolysis" has now become widely current. Unfortunately many zoölogists use the terms cytolysis and plasmolysis interchangeably.

If we define cytolysis as a breaking up of the cell within the membrane or actual liberation of the cell contents, we may define plasmolysis as a shrinkage of the cell contents. Now, certainly hypertonic sea-water as employed in these experiments never caused any liberation of the cell contents. We can not, therefore, regard the action of hypertonic sea-water as cytolytic.

There is another way of reaching a conclusion in this matter. Prolonged exposure to butyric acid in sea-water will cause the uninseminated egg of *Arbacia* on return to normal sea-water to form a fine gelatinous film instead of a membrane. Such eggs, as Loeb noted, soon cytolyze. We may accept this specific instance as a definition. Now, such eggs go to pieces by droplet formation; thus they disintegrate. Or, if eggs with membranes induced by exposure to butyric acid are allowed to lie in normal sea-water, they eventually disintegrate by the formation of globules in the cortex. The disintegration eventually involves the whole egg.

In hypotonic sea-water both *Arbacia* and *Echinarachnius* eggs take up water, lose pigment, and assume a granular appearance. The contents then slowly disappear as if washed away. Rarely do the contents of the eggs burst through the membrane before total disintegration.

Now, the effect of hypertonic sea-water on these eggs is unlike that of butyric acid or hypotonic sea-water. Rather the effect of hypertonic sea-water is plasmolytic. In it the egg shrinks, becomes darker. On return to normal sea-water such an egg, if it fail to develop, remains intact for hours.

Unless, therefore, we change the meaning of the term cytolysis, the hypertonic sea-water employed in these experiments is not cytolytic. Instead of disintegrating, the eggs on return to normal sea-water cleave, gastrulate, and reach the pluteus stage.

3. If we admit that hypertonic sea-water does not call forth membranes by superficial cytolysis, then we must conclude that the hypothesis of a superficial cytolysis as part of the mechanism of experimental parthenogenesis is as unnecessary for a theory of experimental parthenogenesis as it is superficial and inadequate for a theory of fertilization. This must follow for several reasons.

First, we well know from older work that hypertonic sea-water alone is sufficient for the production of plutei. So-called agents of superficial cytolysis do, of course, improve results, but are not absolutely essential. Moreover, for many eggs hypertonic sea-water alone will initiate development; the majority of ova that respond at all to agents that initiate development need but a single agent. The egg of *Arbacia* is no exception. It is entirely unnecessary to use an agent of superficial cytolysis as either a primary or secondary factor for the production of a high per cent. of plutei of great degree of normality.

Secondly, according to Loeb, the agent of superficial cytolysis may be used either before or after the hypertonic sea-water. If butyric acid is as effective after hypertonic sea-water treatment as before, on what logical grounds can we speak of hypertonic sea-water as a corrective factor for the superficial cytolysis yet to take place?

Finally, the hypothesis of a superficial cytolysis as part of the theory of experimental parthenogenesis is untenable because it assigns a rôle to hypertonic sea-water which is the opposite of that of any agent of superficial cytolysis. Since, as shown above, the hypertonic sea-water alone, if of sufficient strength, does just what the butyric acid will do—call forth membranes—the case falls. In order to save the theory, it would be necessary to assume that the hypertonic sea-water of the strength used by the writer to induce membranes has two effects.[1] First, it acts as butyric acid by superficially cytolyzing the egg; and, second, it acts as a corrective factor to correct its first effect. This interpretation in turn entails assumptions which together make it worthy of no serious consideration.

If, for example, we insist that the first effect of hypertonic sea-water is cytolytic, then we must change the connotation of the word cytolysis. Further, the hypertonic sea-water employed by the writer brings about membrane separation while the eggs are *in the solution*. This fact, now, entails an interesting assumption: since following butyric-acid treatment as employed by Loeb the egg of *Arbacia* cytolyzes on return to sea-water, therefore indicating that the butyric acid renders the egg more susceptible to sea-water cytolysis (that is, the acid acts as a catalyst to the ordinary cytolytic action of sea-water on the uninseminated egg), the hypertonic sea-water of the concentrations used by the writer possesses three distinct actions: (1) It prepares the egg for cytolysis as does butyric acid; (2) it cytolyzes as does the normal sea-water following butyric-acid treatment; and (3) it corrects this cytolysis as does the hypertonic sea-water as used by Loeb. Of course, this may well be. It does seem, however, a rather cumbersome suggestion.

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[1] Loeb does make just this assumption. I must confess, though, that I fail to follow his reasoning. See Loeb ("Artificial Parthenogenesis and Fertilization," The University of Chicago Press, 1913, page 159.)

It would thus appear that the hypertonic sea-water being alone sufficient, butyric acid is not necessary. Since, moreover, as I have elsewhere pointed out (Just, '20), there are cogent reasons for the position that butyric acid *does not* cause membrane separation through a superficial cytolysis, the superficial cytolysis-corrective factor hypothesis becomes untenable. Rather it is far more simple to explain the action of butyric acid and of the hypertonic sea-water as used by Loeb as *additive:* together they accomplish what the hypertonic sea-water alone in my experiments accomplishes. The butyric acid-hypertonic sea-water method, beautiful though it is and technically brilliant, confuses the picture because of the superficial cytolysis-corrective factor theory to which it gave rise.

In any field the pioneer work is usually qualitative. The work is none the less important therefor. And yet one can not but feel that it is a pity that Loeb did not make exact observations with various concentrations of salt—particularly so since the method involved is such a simple quantitative one.

If, now, we reject the superficial cytolysis-corrective factor hypothesis as an explanation of experimental parthenogenesis, what explanation do we offer? While it seems to me, in the present state of our knowledge of this subject, far more profitable to collect data than to theorize, it is nevertheless true that the data presented above permit at least a provisional hypothesis. Certainly, we may draw conclusions from the work if these be consistent with the data.

To begin with, it is difficult to conceive of the initiation of development being fundamentally different for different ova. The differences encountered are doubtless merely incidental. Any explanation of experimental parthenogenesis ought, therefore, be congruous—it ought to be applicable to all eggs capable of experimental initiation of development.

But there are serious difficulties in the way of reducing all work on experimental parthenogenesis to a common basis. Leaving out work which is manifestly erroneous, we still have a large body of data purporting to deal with "artificial" parthenogenesis which as a matter of fact merely details results in producing membranes, or some slight cortical change, in initiating maturation, etc. In much of this work indubitable death changes are mistaken for cleavage. And we are told that all these are important for science. And even where experimental parthenogenesis is specifically defined as the production of cell division many substances are named as agents of experimental parthenogenesis, whereas such agents if allowed to act but an extremely short time either call forth membrane separation merely and initiate coagulative death changes. Such results have far less significance for the problem of experimental parthenogenesis than death stiffening for the theory of muscle contraction. We are thus forced to discard much of this work also.

On the other hand, it would be unscientific to reach conclusions for all ova from the results obtained on one. Fortunately, however, we possess many investigations in the field of experimental parthenogenesis of undoubted value. Such, for example, is the work of R. S. Lillie on the egg of *Asterias,* of Miss Allyn's on the egg of *Chætopterus,* in addition, of course, to Loeb's work. Now, in all this work the only common factor is the use of a single agent, heat, butyric acid, or hypertonic sea-water. If we add the eggs of *Nereis* and of the frog to those just mentioned, we have, with respect to the stage in maturation at which fertilization takes place or experimental parthenogenesis is possible, all types of eggs represented. It may be generally true, therefore, wherever experimental parthenogenesis is possible, that a single agent suffices.

In both fertilization and experimental parthenogenesis one fundamental reaction takes place, namely, the cortical reaction. This is no mere arbitrary assumption. Eggs pass through a period of fertilizability. This period is likewise the optimum for experimental parthenogenesis. In some cases this fertilizability we know is due to the presence in or at the cortex of a substance, fertilizin. Complete fertilization-reaction depends upon the combination of fertilizin and sperm. The cortical explosions leading to membrane separation are the sign and sequela of this complete fertilization-reaction. There is evidence that in experimental parthenogenesis also the same phenomena obtain (Lillie, '14, '20[a], '20[b]; Moore, '16, '17; Just, '15, '19[a], '19[b]).

Now, in fertilization the primary object is the incorporation of the sperm nucleus to the end that chromosomes of each parent are alike present in the ensuing division. This object is attained by the reaction between sperm and fertilizin by which the sperm head is made to swell and to form an aster out of aster-forming substance present in the egg. The sperm thus carries the aster to the egg nucleus and cell division ensues.

There is evidence that indicates that the aster-forming substance and fertilizin are not identical, though they may be spatially related. The work of Delage, Wilson Yatsu, and R. S. Lillie shows that in the eggs studied the capacity for merogeny, fertilization, and experimental parthenogenesis depends upon the presence in the cytoplasm of material from the germinal vesicle. We might interpret this to mean that the fertilizability depends upon the presence of fertilizin alone, and that fertilizin and the aster-forming substance are identical. But on this basis how shall we account for fertilization in *Nereis* and *Platynereis?* In these eggs the fertilization-reaction takes place while the egg is in the germinal vesicle stage. At this stage fertilizin is already at the cortex. The sperm aster, on the other hand, never forms until the sperm is in the endoplasm into which germinal vesicle sap has diffused. The sperm aster arises similarly in the eggs of *Chætopterus* and of *Allolobophora.* Where, as in the eggs of *Arbacia* and of *Echinarachnius,* the fertilization-reaction normally takes place in the mature egg, the germinal vesicle material by diffusion has previously reached the ectoplasm; the sperm aster forms, therefore, shortly after the sperm passes the cortex.

In experimental parthenogenesis as in fertilization cell division depends upon the localization of aster-forming substance around the egg nucleus. Optimum localization is enhanced by complete cortical reaction and by exposure to the agent beyond that sufficient for cortical change. (See R. S. Lillie, effect of butyric acid or heat on starfish eggs; Miss Allyn, effect of heat on *Chætopterus* eggs; hypertonic sea-water on urchin eggs as in this paper.)

Instead of the superficial cytolysis-corrective factor theory of experimental parthenogenesis I suggest that the activating agent binds fertilizin, thus leading to complete cortical change. This complete cortical change makes it possible that the additional action of the agent brings about an optimum concentration of aster-forming substance around the egg nucleus. The nucleus swells, a bipolar spindle forms, and development begins.

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INITIATION OF DEVELOPMENT IN THE EGG OF *ARBACIA.*

II. Fertilization of Eggs in Various Stages of Artificially Induced Mitosis.

E. E. JUST,[1]

Rosenwald Fellow in Biology, National Research Council.

A few summers ago the writer made certain experiments with various agents that caused the breakdown of the intact nucleus of the mature uninseminated eggs of *Arbacia* and of *Echinarachnius* with consequent liberation of the chromosomes in the cytoplasm. During the season of 1921 at the Marine Biological Laboratory, Woods Hole, Mass., opportunity presented itself to repeat some of these experiments as part of a detailed study of the effects of sea-water in varying degrees of hypertonicity on the uninseminated *Arbacia* egg. The present paper deals with the results obtained with one concentration only: sea-water made hypertonic by the addition of NaCl or KCl (in the proportion of 8 parts 2½ *M* salt to 50 parts sea-water). This report aims to set forth (1) that exposure to this hypertonic sea-water gives cleavage and a small per cent. of plutei, and (2) that eggs following treatment with this hypertonic sea-water on return to normal sea-water are capable of fertilization during any stage of the first cleavage mitosis except the telophase. It is this second finding which would seem to make this report of some interest: it suggests another method of attacking the problem of fertilization in the egg of *Arbacia*.

I.

The initiation of cell division in the egg of *Arbacia* by means of hypertonic sea-water is too well known to merit more than the briefest description. The pioneer work on this subject is, of course, that of Morgan; it was Loeb, however, who first produced larvæ with this method. Wilson has given an excellent account of the cytology of the egg of *Toxopneustes* induced to develop by hypertonic sea-water. The present study has nothing to add to these and other accounts of experimental parthenogenesis in echinid ova.

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[1] Zoölogical Laboratory, Howard University.

Briefly, I found that among a given lot of eggs in normal sea-water following exposure to hypertonic sea-water some would divide and form plutei that failed to swim at the surface, and that among those eggs that did not show cleavage were some that appeared unaffected by the exposure, while others formed monasters. Prolonged exposure was found to induce numerous cytasters. The egg nucleus, so far as I could determine, undergoes no increase in size while the eggs are in the hypertonic sea-water. I was likewise able to confirm Moore's finding, namely, that these eggs in sea-water after exposure to hypertonic sea-water give off fertilizin. These eggs never form membranes, as is well known.

II.

Moore has shown in an important contribution to the analysis of fertilization that the eggs of *Arbacia* inseminated immediately on return to sea-water after exposure to hypertonic sea-water (in the proportion of 8 parts $2\frac{1}{2}$ *M* NaCl to 50 parts sea-water) develop for the most part in greater numbers than similarly exposed eggs without insemination. These inseminated eggs produce membranes comparable to those found on normally fertilized eggs. Cytological study revealed that sperm penetrate these eggs treated with hypertonic sea-water, but play no active rôle.

For the most part my findings agree with those of Moore. Since, however, the object of my study was somewhat different from his, my methods differed. This difference in method may well account for those of my results that are at variance with his.

In his experiments Moore gave the eggs graded treatment with hypertonic sea-water and inseminated them *immediately* on return to sea-water. In my experiments, since the primary object was to learn the fertilization capacity of the egg of *Arbacia* whose nucleus, having broken down, was thus in one or another stage of mitosis, I inseminated the eggs *at intervals* following their return to normal sea-water. It may be that the egg should lie for a time in normal sea-water, subsequent to exposure to hypertonic sea-water, before insemination in order to regain its equilibrium with the normal medium. If this be true, it would perhaps account for the fact that I obtained much higher percentages of cleavage and of plutei from my inseminations than Moore from his.

Moore, as mentioned above, found scant evidence in his cytological study of any pronounced activity of the sperm. However, I am sure that in my material the sperm is quite active; it may form an aster, swell, and form chromosomes. (Cf. Herbst's studies.)

The method employed in these experiments is simple. Eggs from one *Arbacia* are collected as free from cœlomic fluid as possible, washed, allowed to settle, and exposed to hypertonic sea-water (in the proportion of 8 parts $2\frac{1}{2}\ M$ NaCl or KCl to 50 parts sea-water). After varying lengths of time eggs are removed to normal sea-water. When they have reached the desired stage—monaster, metaphase, etc.—a portion is lightly inseminated with fresh, clean sperm suspension. The development of the inseminated eggs is compared with that of the uninseminated—per cent. of membranes, cleavage, and swimming larvæ. The following table presents the essential results of some of these experi-

TABLE I.

COMPARISON OF THE EFFECT OF EXPOSING EGGS OF *ARBACIA* TO HYPERTONIC SEA-WATER (IN THE PROPORTION OF 8 PARTS $2\frac{1}{2}\ M$ NACL * TO 50 PARTS SEA-WATER) ALONE WITH THAT OF INSEMINATING EGGS IN WHICH NUCLEAR CHANGES HAVE BEEN INDUCED BY HYPERTONIC SEA-WATER.

No. of Experiment.	Time in Minutes of Exposure to Hypertonic Sea-water.	Effect of Exposure to Hypertonic Sea-water as Revealed by Per Cent. of Cleavage and of Larvæ.		Time in Minutes after Exposure to Hypertonic Sea-water when Inseminated.	Nuclear State of Eggs when Inseminated.	Effect of Insemination as Revealed by Per Cent. of Cleavage and of Top Swimming Larvæ.	
		Cleavage.	Larvæ.			Cleavage.	Larvæ (Estimated).
1	40	23	11	152	Swollen nucleus; monaster; spindle.	98	95
2	30	2	5	60	Swollen nucleus; monaster.	96	90
3	30	14	8	45	Monaster; spindle.	90	85
4	81	47	14	65	Spindle; monaster.	93	90
5	60	14	8	80	Spindles; many cytasters.	68	60
6	54	38	15	50	Metaphase; cytasters.	60	40
7	105	8	3	105	Anaphase; telophase.	38	25

* KCl gives similar results.

ments. It should be pointed out that the hypertonic sea-water used in these experiments never gave membranes. Insemination in normal sea-water of eggs that have had treatment with hypertonic sea-water may give 100 per cent. membranes.

We thus see that the egg of *Arbacia* that has such treatment with hypertonic sea-water that mitosis starts up is capable of responding to insemination during any phase of mitosis except the terminal. So far as I have been able to determine, insemination resulting in sperm penetration and perfect membrane separation is as easy to obtain in the anaphase as in the normal resting stage. The plutei from these eggs are as viable as normal plutei.

With the onset of the cortical changes preceding first cleavage, these eggs fail to respond to insemination. So far I have not studied the effect of inseminating these eggs after first cleavage. According to Loeb, however, the blastomeres of the egg of the California sea-urchin induced to cleave through exposure to hypertonic sea-water separate membranes on insemination. Moore, on the other hand, was unable to obtain membranes after inseminating isolated blastomeres of *Arbacia* eggs induced to cleave by exposure to hypertonic sea-water.

I have in my work encountered eggs that simulate first cleavage; in such eggs one of the "pseudo-blastomeres" on insemination will form a membrane, cleave, and swim. The other member of the pair never shows any trace of development. Such eggs seemingly composed of two blastomeres may be easily produced in large numbers.

If eggs that have had an exposure to hypertonic sea-water be shaken gently or squirted through a pipette into normal sea-water, they appear as eggs in the two-cell stage. These pseudo-blastomeres may be equal in size or of all degrees of inequality in size.

Such eggs on insemination form membranes around one component only, regardless of its size. Thus one may get a large or small cleaving egg within a membrane and subsequently a swimming form, attached to an inert mass of cytoplasm without the vestige of membrane. The explanation of this condition is simple.

The hypertonic sea-water so alters the cortex of the egg that it breaks when the egg is shaken and allows an outflow of endoplasm. It is this endoplasm free of cortical material that fails to respond to insemination. The egg nucleus may be located in either of the "pseudo-blastomeres"; or, as is frequently the case, it may be seen lying in the constriction between the "pseudo-blastomeres."

An interesting figure found in sectioned material deserves passing notice. In this case the spindle is in late anaphase; one pole with a chromosome group is in the larger of the "pseudo-blastomeres," which doubtless formed a membrane, since all the eggs in this lot had membranes, and the other pole with a group of chromosomes is in a minute protuberance that could easily pass as a polar body. A spermatozoön was found in the cortex at the pole opposite the "polar body." This picture may be worthy of more than passing comment.

Observations on various ova (*Myzostoma, Chætopterus, Dentalium, Amphioxus, Clepsine,* etc.) show that the ectoplasmic layer is absent at the outer end of the maturation spindle. Moreover, Chambers by microdissection has shown that the region over the outer end of the maturation spindle in the egg of *Cerebratulus* is very fluid. If we assume an absence of cortex in this region (or perhaps physical or chemical difference), polar-body formation would be comparable to this extrusion of endoplasm through the cortex that I have mentioned above.

Observations likewise show that in various ova (those of *Ciona, Cynthia, Chætopterus, Nematodes,* etc.) the cortex as well as the endoplasm flows toward the vegetative pole. In *Chætopterus,* for example, the ectoplasmic waves are clearly visible in the living egg. It is not wholly impossible, therefore, that the definitive location of the maturation spindle at the animal pole is owing as much to the energy of these downward movements as to the movement of the spindle itself. It is as if the egg substance *flows away* from the animal pole, leaving the spindle behind. On this assumption the size of the polar body would thus depend upon the size of the more fluid ectoplasmic defect at the animal pole, the energy of the downward movement of the ectoplasm, or both. Conklin's production of large polar bodies in the egg of *Crepidula* through centrifuging might be cited as evidence that an unusual bulk of material may be thrust out of the egg as a polar body.

Usually polar bodies do not fertilize. In ova in which maturation normally takes place before fertilization this failure to fertilize may be due to absence of fertilizin. If normally fertilization occurs during first maturation, the failure of polar bodies to fertilize may be due to the fixation of fertilizin; polar bodies are thus sterile as are all parts of the egg. If it prove that polar bodies lack cortex and are really endoplasmic, this would be suggestive as to the location of fertilizin in such ova, as, for example, *Asterias,* in which fertilization may take place at various stages of maturation.

III.

The experiments above noted show that eggs of *Arbacia* induced through treatment with hypertonic sea-water to initiate mitosis after return to sea-water are capable of giving a response to insemination similar to that in normal fertilization. This gives rise to several considerations. We may discuss these in turn.

1. In the first place, the stage in mitosis through which an egg is passing at the time of insemination is of no consequence for complete cortical response. Eggs in any stage of mitosis (except the telophase) respond completely to insemination whether or not the spermatozoa entering such eggs take part in the ensuing divisions. This would indicate that physical or chemical changes set up in the cytoplasm during mitosis constitute no bar to fertilization. In this respect changes set up by the first cleavage mitosis do not differ from those in the maturation mitoses in those eggs that normally take in sperm before complete maturation. Thus changes in permeability, rate of oxidation—themselves held as "causes" of fertilization—do not interfere with the cortical reaction to sperm.

If we define fertilization, in terms of this cortical phenomenon, as an instantaneous reaction between some ovogenous substance and the spermatozoön at the time of insemination, the experiments here reported are again suggestive. Of course, the reader may not accept this definition of fertilization; indeed, it may turn out to be wholly fallacious. Let us, however, for the sake of argument, assume that the definition is correct; that the primary aim in the fertilization process is the incorporation of the sperm head as part of the zygote nucleus, thus insuring equivalence of maternal and paternal chromatin in heredity; and that the reaction at the egg cortex between fertilizin and sperm guarantees this incorporation. Cell division is thus the end result of fertilization. On this hypothesis, then, the experiments here reported suggest a mode of attacking the fertilization-reaction apart from cell division.

In the third place, our results suggest the possibility of testing the validity of various theories of fertilization: for example, the oxidation (Loeb), the permeability (R. S. Lillie, McClendon, Gray), and the viscosity (Fischer and Ostwald, Heilbrunn) theories. If, after treating *Arbacia* eggs with hypertonic sea-water of the strength used in the experiments cited above, we were to find *no* increase in permeability or oxidation, for example, but on inseminating these eggs were to find *pronounced* increase, the case for such increase as the "cause" of fertilization would demand a hearing. But hypertonic sea-water alone of the strength used in our experiments increases the rate of oxidation and permeability. Moreover, this being true, even if there were an additional increase in the rate of oxidation (or permeability) following the insemination of these eggs previously exposed to hypertonic sea-water, and if this increase plus that due to hypertonic sea-water alone were equal to the increase of an equal number of eggs from the same female following normal insemination, we could not hold the oxidation (or permeability) theory as proved. For the fact still remains that oxidation (or permeability) increase is not inseparably bound up with the fertilization-reaction.

The case is similar with the viscosity theory of fertilization. Mrs. Andrews long ago showed by means of subjecting eggs to pressure that a rhythm of viscosity changes accompanies the cleavage process. It may also well be that following the liquefaction of the cortex, as in the egg of *Echinarachnius,* the ectoplasm gels. Also, Lillie finds that in unfertilized eggs of *Chætopterus* in the mesophase of first maturation, though stratification of the endoplasm readily results from centrifuging, the ectoplasmic layer remains unaffected. That fluid substance diffuses from nucleus into cytoplasm is of course well known. This has been shown especially for the egg in the germinal vesicle stage. Says F. R. Lillie on this point: "During this period of diffusion of the fluid substance of the germinal vesicle and the ensuing polarization of the ectoplasm and endoplasm, the protoplasm as a whole possesses a much higher degree of fluidity than before" (Lillie, '06, page 176). "The strongest evidence for greater fluidity at this time is found in the fact that the ectoplasmic spherules are much more numerous and smaller than they were previously or than they are subsequently. Evidently there is a reversible process of coagulation concerned, the spherules breaking down into smaller particles as the fluidity increases and setting or coagulating again by a process of fusion."

"If eggs are allowed to stand eight to fifteen minutes in sea-water after being taken from the female so that in some the germinal vesicle is intact and in others broken down, the latter always show stratification more or less pronounced after centrifuging and the former never show it. . . . It would seem that the endoplasm has become less viscid as a result of the diffusion of substance from the germinal vesicle so as to permit of a closer aggregation of the yellow granules."

Let us, however, waiving this demonstration of a difference in the physical make-up of the cortex and endoplasm of the *unfertilized Chætopterus* egg, as well as Chambers's observation that the gelation succeeding insemination is localized in the region of the sperm aster, assume that in the *Arbacia* egg a gelation follows the liquefaction of the cortex. But the viscosity theory of fertilization is not thus made more tenable.

According to the viscosity theory (Heilbrunn), initiation of development by artificial agents or by sperm involves coagulation of the eggs; the mitotic spindle probably arises as a direct result of this coagulative change. But in the experiments cited above in this paper eggs that have first cleavage spindles (cf. many eggs normally fertilized during maturation phases) respond to insemination. Such eggs will develop without the cortical changes that in normal eggs follow insemination. We should, however, point out that, according to Heilbrunn, hypertonic sea-water coagulates the egg.[1] It may thus be argued that those eggs with spindles are only incompletely activated if they give cortical response to insemination. The truth, though, is quite otherwise. The cortical changes induced by sperm are not merely quantitatively but qualitatively different from those induced by hypertonic sea-water; the cortex is liquified following insemination with resulting membrane separation, but it is thickened by hypertonic sea-water. If anything were wanting to substantiate the view here presented, Heilbrunn's own findings would do so: many diverse agents besides hypertonic sea-water produce coagulation in the egg. That Heilbrunn does not show that all of these so-called agents of artificial parthenogenesis do actually produce cell division—and this according to his own definition of artificial parthenogenesis they should do—alone is fatal for the whole theory. In addition, it is not beyond the realm of possibility that many of these agents (distilled water acting upward to *four minutes,* toluene acting for *five minutes,* saponin acting for *five minutes,* etc.) simply induce death changes—of no significance whatsoever for the problem of fertilization.

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[1] All that Heilbrunn shows is that the eggs are more viscous while *in hypertonic solutions.* We are told nothing of their history after return to normal sea-water.

There remains the fertilizin theory (Lillie, '14). Here we find no difficulty. Eggs of *Arbacia* following exposure to hypertonic sea-water possess fertilizin despite mitotic changes. Such eggs on insemination, as we should expect, give complete cortical reaction. Without insemination such eggs develop. *Fertilizin fertilizes the sperm* so that it may by reacting with aster-forming substance of the egg start up division in which the egg nucleus takes part. But eggs that are already induced to develop by hypertonic sea-water do so through localization of aster-forming substance around their nuclei. Sperm entering such eggs may still react with fertilizin, thereby setting free cortical changes leading to membrane separation.

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INITIATION OF DEVELOPMENT IN THE EGG OF *ARBACIA*.

III. The Effect of *Arbacia* Blood on the Fertilization-Reaction.

E. E. JUST,[1]

Rosenwald Fellow in Biology, National Research Council.

I.

During May, 1921, *Arbacia* examined at Woods Hole, Mass., were found to be immature. Thus, on May 15, bits of the gonads examined under the microscope contained only immature eggs devoid of color. The testes showed no mature sperm. Toward the end of May a few females were found in which the eggs were undergoing maturation. Early in June (June 1 to 8) the eggs gave very low per cent. fertilization. From this time on eggs exuded through the genital pores steadily increased in fertilizability. Eggs taken from the ovaries, on the other hand, were largely immature; their fertilization per cent. was lower than that of eggs that exuded from the genital pores when the animals were carefully cut around the peristome. Toward the end of July the high fertilization capacity of the shed eggs (*i.e.,* eggs that exuded from the genital pores when the animals were carefully cut around the peristome) fell off. From July 20 to the end of the season the fertilization capacity of shed eggs from a given female was found to be markedly inferior to eggs taken from the same female by cutting up the ovaries in sea-water. There was thus found during the first part of the season in the case of shed eggs a rise in fertilizability followed by a fall. Parallel with this fall was found a rise in fertilizability of eggs from the ovaries which during June were largely immature. It was thus found that eggs of *Arbacia* of high fertilization capacity may be obtained at Woods Hole throughout the season provided shed eggs are used during the early part and eggs from the ovaries during the later part of the season. Evidence for these statements is included in this paper.

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[1] Zoölogical Laboratory, Howard University.

In what follows it will be convenient to consider first those experiments made on shed eggs during the first period of the summer, and second those experiments made on both shed and "ovary" eggs during the second part of the summer.

II.

A word concerning the method used in the experiments here reported may not be amiss. Eggs used were from freshly procured animals whenever these were to be had. In one case animals were collected from the spiles of the dock opposite the laboratory and used immediately after. Results on eggs from animals kept in the laboratory in a few instances for several days, however, showed that such eggs may be as good as those from freshly dredged animals. Each animal was thoroughly cleansed for a minute or two under running tap water. The hands and forearms of the worker were kept under a stream of tap water for about one minute. After the washing under running tap water the urchin was shaken dry and put under a jet of sea-water, then shaken dry again. The animal was thereupon placed in a dry Syracuse watch glass, aboral side down, and its peristome carefully cut with clean, sharp-pointed scissors with very slender blades. Care was exercised to avoid puncturing the gonads. Frequently mere puncture of the peristome was made. As soon as eggs or sperm came through the genital pores as a result of the stimulus of the incision the animal was removed to another dish. Controls of uninseminated eggs were always run and not once was development of an egg discovered. The case may be quite otherwise, however, if the animals are not thoroughly washed in tap water. Among eggs from such unwashed females one may find not only eggs with membranes, but cleavage stages and blastulæ. This is particularly true of animals kept in the tanks where spawning may take place. In such cases eggs caught among the female's spines are fertilized where they remain until the swimming stage.

The blood used in this work was procured in the following manner: The peristome was carefully cut away and the lantern gently removed. The animal was then inverted over a clean Syracuse watch glass and drained of fluid, removed to another watch glass, and allowed to shed. The blood from each animal was used separately or that from several animals united. After clotting, the serum was decanted and set aside until needed. Sea-water in which blood clots were cut up after filtering was used in one experiment. It seemed no different in its action from the serum.

A.

We may begin with the experiments which indicate that during the early part of the season *Arbacia* eggs that exude through the genital pores are of high fertilization capacity. Blood added to these eggs before insemination cuts down the per cent. of fertilization.

The Experiments.

(*a*) July 14, 4:00 P.M. 1 drop of shed eggs from one female in 10 c.c. of sea-water allowed to settle. Water changed three times. Eggs inseminated—Lot *A*.

2 drops of shed eggs from same female in 5 c.c. of sea-water plus 5 c.c. of blood. Allowed to settle. 1 drop of these eggs removed to 10 c.c. of sea-water—Lot *B*—and inseminated. Remainder of the same eggs inseminated—Lot *C*.

7:00 P.M. These eggs give cleavage as follows:

Lot.	*A*.	*B*.	*C*.
Per cent. of cleavage	98	2	0

This experiment is typical of a group of experiments made during the month of June and the first three weeks of July. I always found some inhibitor present in the blood of females, though the amount might vary to a considerable degree. Thus in some cases, particularly in the early experiments, a great deal of blood from several animals had to be used to inhibit fertilization. These experiments, without a single exception, reveal a high capacity for fertilization on the part of shed eggs.

(*b*) July 16, 8:00 P.M. 4 drops of shed eggs from each of three females (*A*, *B*, and *C*) distributed as follows:

*A*1, *B*1, *C*1: 1 drop of shed eggs inseminated in 250 c.c. of sea-water.

A2, *B2*, *C2*: 1 drop of shed eggs plus 2 drops of blood inseminated in sea-water.

A3, *B3*, *C3*: 1 drop of shed eggs plus 4 drops of blood inseminated in sea-water.

A4, *B4*, *C4*: 1 drop of shed eggs plus 8 drops of blood inseminated in sea-water.

10:00 P.M. *A1*, *B1*, *C1* show 96, 98, and 93 per cent. fertilization, respectively. No development in other dishes.

July 17, 10:30 A.M. Nos. *A2*, *B2*, and *C2* show about .01 per cent. development. No development in the others.

Nos. *A2* to *C4*, inclusive, washed and set aside. 3:00 P.M. No cleavage. Samples from each of these inseminated. 3:10 P.M. Good membranes in most eggs of Nos. *A2* to *C3*, inclusive. Nos. *A4* to *C4* only about 40 per cent. membranes each.

3:45 P.M. First cleavage in all eggs that have membranes.

7:30 P.M. Other samples from the eggs *A2* to *C4*, inclusive, which were washed this morning inseminated.

9:00 P.M. About .01 per cent. in two-cell stage in *B2*. No development in others, though numerous sperm attached. All dishes show active sperm.

July 18, 8:00 A.M. One blastula in *B2*.

This experiment and others of this group show several points. They show that shed eggs of high fertilization capacity will not fertilize in the presence of blood. The amount of blood necessary to bring about this inhibition can not be predicted because the inhibitory power of the blood of various females is not exactly the same. In this group of experiments, however, made usually with eggs from different females in samples of the same blood, the results are fairly constant; the inhibition to fertilization is about the same. Finally, the experiment indicates that twenty-six and one half hours after insemination in the presence of blood eggs may on reinsemination be made to develop. Usually such capacity to respond to reinsemination does not persist after twenty-four hours. As Lillie has shown, the presence of blood does not interfere with the action of the sperm agglutinin (fertilizin) produced by *Arbacia*. It would have been interesting to compare the production of fertilizin by these eggs inseminated in blood with that of an equal quantity of uninseminated eggs from the same female in normal sea-water. Unfortunately this was not done.

(*c*) July 18, 10:00 A.M. 0.5 c.c. of shed eggs (sample of which on insemination gave 98 per cent. membranes) in 10 c.c. of blood—Lot *A*. Following series set up:

No. 1. 5 c.c. of Lot *A* plus 5 c.c. of sea-water.
“ 2. “ “ No. 1 “ “ “ “ “
“ 3. “ “ No. 2 “ “ “ “ “
“ 4. “ “ No. 3 “ “ “ “ “
“ 5. “ “ No. 4 “ “ “ “ “
“ 6. “ “ No. 5 “ “ “ “ “
“ 7. “ “ No. 6 “ “ “ “ “

All inseminated heavily.

12:00 M. Cleavages in these eggs as follows:

No.	2.	3.	4.	5.	6.	7.
Per cent. of cleavage...............	0	0	0	10	20	25

Nos. 2, 3, and 4 killed and sectioned.

The interesting point here is that despite the graded decrease in the quantity of both eggs and blood the inhibitory action of the blood persists. Each lot of eggs was inseminated with the same amount of sperm. This means that the heaviness of insemination steadily increased in the Nos. 2 to 7, for the number of eggs was halved in each successive dilution. It would seem, therefore, that heavy insemination does not necessarily mean an overcoming of the blood block to fertilization. That the failure to cleave was not due to polyspermy we may conclude from the per cent. of cleavage in Nos. 5, 6, and 7, which received relatively to the number of eggs most heavy insemination. However, I should point out that this was the most powerful inhibiting blood encountered in this group of experiments (made during June and to July 20).

(*d*) July 19, 6:30 P.M. 10 c.c. suspension of shed eggs (sample of which gave 98 per cent. membranes) plus 10 c.c. of blood inseminated.

9:00 P.M. No development. Eggs washed in 250 c.c. sea-water. Set aside; on settling, removed to 250 c.c. of sea-water.

July 20, 8:00 to 9:00 A.M. These eggs show a very small per cent. fine top swimmers. Undeveloped eggs show sperm attached. Top swimmers removed. 250 c.c. of fresh sea-water added to remaining eggs.

10:20 A.M. About 1 per cent. of eggs in the 2- and 4-cell stages.

10:25 A.M. Eggs inseminated in 250 c.c. of fresh sea-water.

11:25 A.M. 5 per cent. of eggs in 4-cell stage, 85 per cent. in 2-cell stage. Some others show spindles, remainder no change. Eggs tend to lose membranes and to bud.

4:00 P.M. Small per cent. of eggs in swimming stage.

6:00 P.M. About 90 per cent. swimming.

9:00 P.M. Swimmers at the surface.

The chief point of interest in this experiment is that eggs inseminated in blood may, on washing, develop normally. If portions of such eggs are washed at intervals up to about two hours after insemination, a high per cent. develop perfectly. They lose their power to develop on washing two to three hours after insemination. This seems to be the most significant point of this report (see beyond, page 421). Eggs inseminated in the presence of blood take in sperm; such sperm lie within the cortex. All these eggs show sperm firmly attached to the cortex. These eggs have been studied not only while living, but also in sections and *in toto* mounts properly fixed (Bouin, Lang, Meves, Flemming). In some cases the sections show the egg cortex literally studded with sperm, with other sperm within the egg. The failure of these eggs inseminated in blood to develop is not, therefore, due to failure of sperm attachment or penetration. On washing within about two hours after insemination in the presence of blood the eggs are released from the blood block. The per cent. of eggs that develop depends upon the thoroughness of washing within this two-hour limit as well as the amount of inhibitor present.

This experiment likewise reveals that twenty-eight hours after their insemination in the presence of blood eggs, after washing, will fertilize with a high per cent. of development.

B.

We may now consider those experiments which reveal that during the later half of the season the shed eggs of *Arbacia* are of low fertilization capacity, whereas eggs taken from the ovaries are of high fertilization capacity.

The Experiments.

(*a*) July 23, 8:00 A.M. Portions of shed eggs from 12 *Arbacia* inseminated in turn. All poor; not more than 10 per cent. membranes in any lot.

Ovaries from these same females chopped up separately in sea-water and eggs strained out. Eggs inseminated. Fine membranes (average: around 96 per cent.).

This experiment scarcely needs comment. The eggs that exude through the genital pores are inferior to those procured by cutting up the ovaries in sea-water. And this was found to be true from July 23 to the end of the season in 107 out of 125 females tested on this one point alone.

(*b*) July 25, 9:00 A.M. 9 females opened. Eggs exude through genital pores into dry dishes. 250 c.c. of sea-water added to each lot of eggs. Samples from each inseminated. No membranes in Nos. 1, 2, 4, 6, and 7. 80 per cent. membranes in No. 3. Nos. 5, 8, and 9 give 18, 6, and 14 per cent. membranes, respectively. Effect not due to sperm for inseminations were next made in each case with sperm from 7 different males with essentially the same results.

Eggs removed from these 9 females inseminated in turn. Fine membranes; average around 98 per cent.

To offset any criticism that these results might be due to presence of blood with sperm, great care was exercised in opening all animals. During several days, for example, all instruments were sterilized before using by heating red hot in order to destroy any adherent blood. If animals cut proved to be males, they usually exuded clean sperm into the dry watch glasses. In many cases the males shed spontaneously. Thus only uncontaminated sperm was procured. In addition, sperm from several males was used as mentioned in the experiment cited above. Finally, the results of inseminating the eggs taken from the ovaries would seem to offset the possibility that the failure of the shed eggs to cleave is due to the presence of blood with the sperm.

(*c*) July 27. Samples of shed eggs from 14 females inseminated in turn. Very high amount of inhibition present in each of Nos. 1 to 11, inclusive. Membrane separation in these eggs as follows:

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Per cent. of membranes. .	3	5	0	1	6	8	15	0	4	1	0.1	99	98	100

Remainder of uninseminated eggs of Nos. 1 to 11, inclusive, put in one dish. Washed in five changes of 250 c.c. of sea-water. Inseminated. 13 per cent. cleavage.

Ovaries from these same females chopped up separately in sea-water; eggs strained and collected. Portion of each inseminated. Average fertilization—95 per cent. Remaining eggs added and inseminated in one dish. Fertilization—97 per cent.

Eggs from Nos. 12, 13, 14, added, inseminated. Fertilization close to 100 per cent.

These uninseminated shed eggs gave high agglutinin test. I thought it worth while to add the eggs from several females in order to increase the amount of sperm agglutinin (fertilizin); this, however, failed to improve results. These eggs do not fail to develop because of lack of fertilizin or of failure of sperm entry. They are loaded with blood inhibitor and this suspends the fertilization-reaction.

(*d*) July 30, 9:50 A.M. 9 females opened, gently drained of blood. Each lot of shed eggs then in finger bowls of 200 c.c. of sea-water. Portions of these eggs removed and inseminated at 10:00 A.M., 10:28 A.M., 10:43 A.M., and 11:25 A.M., with the following results:

No.	Amount in c.c. of Shed Eggs at 9:50 A.M.	Per Cent. Membranes Following Insemination.			
		10:00 A.M.	10:28 A.M.	10:50 A.M.	11:25 A.M
1.....	3	0	40	60	95
2.....	.1	3	3	Cytolysis	
3.....	−.1	0	[1]	[1]	
4.....	.2	5	90	98	
5.....	.1	0	70	88	
6.....	.1	0	80	90	
7.....	−.1	6	[1]	[1]	
8.....	.15	10	30	97	
9.....	−.1	0	35	95	

[1] Not sufficient eggs left to make count.

11:48 A.M. Ovaries of each of above females (Nos. 1 to 9, inclusive) separately chopped up in sea-water; strained. Each lot of eggs in 200 c.c. of sea-water.

11:55 A.M. Portions of each lot of eggs inseminated.

2:00 P.M. Beautiful cleavage in all lots—close to 100 per cent.

At times the quantity of eggs that exude from the genital pores is really enormous for the size of the urchin. Such eggs are perfectly beautiful. And yet the bulk of the eggs is no criterion for their fertilizability. I have used eggs from animals fresh from the sea. The results are the same; they are not due to the fact that the animals have deteriorated in the laboratory.

The protocols herewith cited (Sections *A* and *B*) constitute the evidence for the conclusions that eggs of *Arbacia* vary with respect to their fertilization capacity during the summer; during the first part of the season shed eggs possess high fertilization capacity which drops off during the latter part of the season; eggs from the ovary, during the latter part of the season when the shed eggs are poor, possess high fertilization capacity. A simple method is thus indicated for obtaining throughout the summer eggs of high fertilizability. The evidence likewise admits of the conclusion that blood blocks fertilization in shed eggs of high fertilizability. It is likewise true, though no experiments have been cited on this point in the present paper, that fertilization of eggs from the ovary with high fertilization capacity is blocked by blood. The findings of the writer as to this effect of blood on eggs of *Arbacia* are thus in accord with those of Lillie ('14).

The experiments cited above also show that eggs in the presence of blood agglutinate to themselves and even take in sperm, and that such sperm may activate at any time during a dormant period of about two hours if the blood is removed by washing. Finally, the experiments reveal that as long as twenty-eight hours after insemination in the presence of blood eggs failing to develop may do so on reinsemination. In view of Lillie's recent work ('21) on the effect of copper salts in fertilization—especially important in revealing the latent period in the fertilization-reaction—these findings of mine may deserve notice since they lend additional support to Lillie's fertilizin theory.

The protocols here given do not, however, establish that blood is responsible for the failure during the latter part of the season of shed eggs to fertilize. The failure of these eggs to fertilize may, indeed, be due to other causes. Nevertheless the following points warrant consideration for the assumption that blood is responsible for this inhibition:

First, the failure of fertilization is not due to the absence of fertilizin. For eggs in blood liberate fertilizin (Lillie, '14).[1] Secondly, washing removes the inhibition to fertilization, and this is true of those eggs whose failure to fertilize is indubitably due to the presence of blood. Thirdly, the inhibition decreases after the eggs have remained in sea-water for some time. And, finally, I have observed during several seasons that late in August *Arbacia* females are frequently turgid with eggs from ruptured ovaries. Such blood-soaked eggs are of low fertilization capacity. These considerations point to a blood block, but they certainly do not prove the case.

Indeed, Oshima, likewise working with the egg of *Arbacia* in September, 1921, after I had left Woods Hole, has interpreted the failure of eggs escaping through the genital pores of opened sea-urchins to fertilize as due to what he calls a "dermal secretion." So far as I can determine, Oshima's *sole* criterion for calling this substance a "dermal secretion"—he could get very little of it from *dermal tissues themselves,* it seems—is the fact that he got it from the outside of the urchins. By the same token, eggs and sperm that exude through the genital pores and lodge among the spines—an observation that every worker with sea-urchins has at some time made—are dermal secretions! Oshima's "dermal secretion," I very much suspect, is an *excretion,* if not actually fecal material. And this suspicion is strengthened by the fact, which Oshima points out, that uric acid is found in it.

Moreover, against Oshima's interpretation that the "dermal secretion" inhibits fertilization we have Lillie's extensive experiments to the contrary. I believe with Oshima that he would have reached an entirely different conclusion had he been able to "carry out this series of experiments more fully and accurately." I heartily concur with his conclusion that the action of the "dermal secretion" seems to have no biological significance.

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[1] I have additional evidence on this point.

III.

Blood blocks fertilization in the egg of *Arbacia*. Lillie's experiments show this. The findings reported above but confirm his.

If we inquire as to the mode in which blood blocks fertilization, we must recall the rôle of fertilizin in the fertilization-reaction. That the fertilizability of the egg depends upon the presence of fertilizin, the following facts show: Immature eggs, fertilized eggs, and eggs with butyric acid membranes do not secrete fertilizin. They are also incapable of fertilization. Fertilizable eggs washed free of fertilizin lose their capacity for fertilization. But the failure of eggs in the presence of blood does not place these eggs in the same category with fertilizin-free eggs.

The failure of eggs in blood to fertilize is not due to the blocking of the reaction between sperm and fertilizin. It will be recalled that the presence of fertilizin in sea-water is indicated by its agglutinative action on specific sperm. This action is not lost in the presence of blood. "It can not be supposed," says Lillie, "that the plasma operates by preventing the adhesion of the spermatozoön to the egg if this is brought about by agglutination, because it was found that the agglutination of spermatozoa by means of egg secretions takes place as readily in the plasma as in sea-water" (Lillie, '19, page 175). The action of blood is thus on the fertilizin; it constitutes a block between fertilizin and the egg itself. Since eggs of *Arbacia* inseminated in blood are found with sperm attached to or within the cortex, it must be concluded that Lillie's interpretation of the inhibitory action of blood is sustained.

Finally, Lillie's copper experiments ('21) reveal the latent period in the fertilization reaction. If it prove that blood acts similarly, we may have an additional method for study of the latent period. We may thus be able more closely to approach an understanding of the fertilization-reaction.

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